

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
25 September 2003 (25.09.2003)

PCT

(10) International Publication Number
WO 03/077947 A1

- (51) International Patent Classification⁷: **A61K 39/395, C07K 16/28**
- (21) International Application Number: **PCT/US03/07647**
- (22) International Filing Date: **13 March 2003 (13.03.2003)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
60/364,513 15 March 2002 (15.03.2002) US
- (71) Applicant: **SCHERING CORPORATION [US/US];**
2000 Galloping Hill Road, Kenilworth, NJ 07033-0530 (US).
- (72) Inventors: **CHERWINSKI, Holly, M.,;** 17100 Cougar Rock Road, Boulder Creek, CA 95006 (US). **PHILLIPS, Joseph, H.,;** 1511 Walnut Drive, Palo Alto, CA 94303 (US). **SEDGWICK, Jonathon, D.,;** 365 North California Avenue, Palo Alto, CA 94301 (US). **BIGLER, Michael, E.,;** 3624 Highland Avenue, Redwood City, CA 94062 (US). **MURPHY, Craig, A.,;** 267 Curtner Avenue, Palo Alto, CA 94306 (US).
- (74) Agent: **DAVIS, Michael, D.,;** Schering-Plough Corporation, Patent Department-K-6-1 1990, Kenilworth, NJ 07033 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LU, LV, MA, MD, MG, MK, MN, MX, MZ, NI, NO, NZ, PH, PL, PT, RO, RU, SC, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UZ, VC, VN, YU, ZA, ZM.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Declaration under Rule 4.17:**
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*
- Published:**
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 03/077947 A1

(54) Title: METHODS OF MODULATING CD200 RECEPTORS

(57) Abstract: ABSTRACT Provided are methods for modulating activity of the immune system using agonists or antagonists of a CD200 receptor. Also provided are methods of treatment and diagnosis of immune disorders.

METHODS OF MODULATING CD200 RECEPTORS

FIELD OF THE INVENTION

5

The present invention relates to methods and compositions for modulating mammalian physiology, including immune system function. In particular, it provides methods for modulating the metabolism and activity of mast cells. Diagnostic and therapeutic uses are disclosed.

10

BACKGROUND OF THE INVENTION

15

The immune system, composed of cells of the bone marrow, spleen, and hematopoietic cells, including but not limited to lymphoid and myeloid lineage cells, is responsible for defending against bacteria, viruses, and foreign multicellular organisms, as well as cancer cells. Improper regulation of the immune system can result in a number of disorders or pathological conditions, e.g., chronic inflammation, autoimmune diseases and disorders, and undesired allergic reactions to foreign particles or foreign tissues.

20

Mast cells, a myeloid lineage immune cell, secrete a variety of cytokines and enzymes that result in inflammation. As some of these substances occur in secretory vesicles that appear granular, the process of secretion is sometimes called degranulation. Rapid degranulation by mast cells contributes to the pathology of asthma, anaphylaxis, and other allergic responses, while slower degranulation by mast cells contributes to arthritis and other types of chronic inflammation. The release of inflammatory cytokines and enzymes by mast cells can result in tissue damage, further attraction of mast cells, resulting in further tissue damage.

25

Cells of the immune system possess many types of membrane-bound proteins that can serve as receptors. The ligands for these receptors can be small molecules, proteins, e.g., cytokines or chemokines, or membrane-bound proteins residing on a separate cell. Changes in the activity of a cell or tissue can result from occupation of a receptor by its physiological ligand, by an analogue of the physiological ligand, by an antibody, by agents that cross-link like-receptors to each other, and by agents that cross-link non-identical receptors to each other.

30

Mast cells contain a number of receptors that relay an inhibitory signal to the cell. These include CD200 receptor a (a.k.a. CD200Ra; OX2Ra), as well as various Ig-ITIM-bearing receptors, e.g., low affinity IgG receptor FcγRIIB, transmembrane glycoprotein receptor gp49B1, signal regulatory protein (SIRP), mast cell function-associated Ag, and platelet

endothelial cell adhesion molecule-1 (PECAM-1)/(CD31) (Wong, et al. (2002) J. Immunol. 168:6455-6462).

CD200 (a.k.a. OX2) is a widely distributed membrane-bound protein occurring on lymphoid, neuronal, endothelial, dendritic cells, and B cells (Wright, et al. (2000) Immunity 13, 233-242; Wright, et al. (2001) Immunology 102:173-179; Hoek, et al. (2000) Science 290:1768-1771; Barclay, et al. (2001) Immunol. 102:173-179; McCaughan, et al. (1987) Immunogenetics 25:329-335). CD200, the ligand of CD200R, can bind to CD200R, which is expressed on a separate cell. In humans, two subtypes of CD200Rs have been identified, hCD200Ra (SEQ ID NO:2) and hCD200Rb (SEQ ID NO:4). Murine homologs of CD200R consist of four receptor subtypes, CD200Ra (SEQ ID NO:6), CD200Rb (SEQ ID NO:8), CD200Rc (SEQ ID NO:10), and CD200Rd (SEQ ID NO:12). CD200Ra occurs, e.g., on macrophages, dendritic cells, and microglia, of the rat (Wright, et al. (2000) supra; Preston, et al. (1997) Eur. J. Immunol. 27:1911-1918).

Several regulatory pathways involving the membrane bound proteins for various immune cells have been identified. However, the molecules responsible for mast cell regulation are poorly understood. The present invention fulfills this need by providing methods of diagnosis and treatment of mast cell disorders by targeting mast cell receptor molecules, e.g., CD200Rs.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows secretion of β -hexosaminidase versus concentration of degranulation signal. Figure 2 shows cytokine release versus concentration of degranulation signal.

SUMMARY OF THE INVENTION

5 The invention is based, in part, upon the discovery that binding of an antibody to an inhibiting receptor, e.g., CD200Ra, inactivates a cell.

 The invention provides a method of modulating the activity of a cell comprising contacting the cell with a binding composition derived from the antigen binding site of an antibody that specifically binds to CD200Ra (SEQ ID NOs:2 or 6), or an antigenic fragment
10 thereof. Also provided is the above method wherein the cell is a mast cell; wherein the modulating inhibits cell activity or stimulates cell activity; wherein the modulating inhibits cell activity and the binding composition comprises an agonist of CD200Ra (SEQ ID NOs:2 or 6); or wherein the modulating increases cell activity and the binding composition comprises an antagonist of CD200Ra (SEQ ID NOs:2 or 6). In another embodiment, the invention provides
15 the above method wherein the binding composition comprises a humanized antibody; a monoclonal antibody; a polyclonal antibody; an Fab fragment; an F(ab')₂ fragment; a peptide mimetic of an antibody; or a detectable label. Yet another aspect of the invention is the above method, further comprising contacting the cell with an agent that specifically enhances expression of CD200Ra (SEQ ID NOs:2 or 6).

20 Also encompassed is a method of treating a subject suffering from an immune condition comprising treating with or administering the binding composition derived from the antigen binding site of an antibody that specifically binds to CD200Ra (SEQ ID NOs:2 or 6), or an antigenic fragment thereof. Also provided is the above method, wherein the binding composition comprises an agonist or antagonist of CD200Ra (SEQ ID NOs:2 or 6); wherein
25 the immune condition is an inflammatory condition or an autoimmune condition. Also contemplated is the above method wherein the immune condition is rheumatoid arthritis; endotoxemia; psoriasis; or allergy; or where the immune condition is an infection or a cancerous condition. Further contemplated is the above method wherein the binding composition is administered in conjunction with an agent that specifically enhances expression
30 CD200Ra (SEQ ID NOs:2 or 6), or an antigenic fragment thereof.

DETAILED DESCRIPTION

As used herein, including the appended claims, the singular forms of words such as "a,"
"an," and "the" include their corresponding plural references unless the context clearly dictates
5 otherwise.

I. Definitions.

"Activity" of a molecule may describe or refer to binding of the molecule to a ligand or
to a receptor, to catalytic activity, to the ability to stimulate gene expression, to antigenic
10 activity, to the modulation of activities of other molecules, and the like. "Activity" of a
molecule may also refer to activity in modulating or maintaining cell-to-cell interactions, e.g.,
adhesion, or activity in maintaining a structure of a cell, e.g., cell membranes or cytoskeleton.
"Activity" may also mean specific activity, e.g., [catalytic activity]/[mg protein], or
[immunological activity]/[mg protein], or the like.

15 "Amino acid" refers to naturally occurring and synthetic amino acids, as well as amino
acid analogs and amino acid mimetics that function in a manner similar to the naturally
occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code,
including selenomethionine, as well as those amino acids that are modified after incorporation
into a polypeptide, e.g., hydroxyproline, O-phosphoserine, O-phosphotyrosine,
20 γ -carboxyglutamate, and cystine. Amino acid analogs refers to compounds that have the same
basic chemical structure as a naturally occurring amino acid, i.e., an α -carbon that is bound to a
hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine,
methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups
(e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure
25 as a naturally occurring amino acid. Amino acid mimetic refers to a chemical compound that
has a structure that is different from the general chemical structure of an amino acid, but that
functions in a manner similar to a naturally occurring amino acid. Amino acids may be
referred to herein by either their commonly known three letter symbols or by their one-letter
symbols.

30 "Binding composition" encompasses, e.g., an antibody, polyclonal antibody,
monoclonal antibody, engineered antibody, recombinant antibody, humanized antibody,
binding fragment derived from an antibody, peptide mimetic of an antibody, a bifunctional, or
multifunctional reagent. The binding composition may further comprise, e.g., a linker,

oligosaccharide, or label. "Derived from an antibody" refers, e.g., to treatment or manipulation of an antibody to produce a fragment or complex, e.g., an antigen-binding site of the antibody, or use of genetically engineering to produce a molecule or complex that mimics predetermined features of the antibody, e.g., the antigen-binding site.

5 "Bispecific antibody" generally refers to a covalent complex, but may refer to a stable non-covalent complex of binding fragments from two different antibodies, humanized binding fragments from two different antibodies, or peptide mimetics of binding fragments from two different antibodies. Each binding fragment recognizes a different target or epitope, e.g., a different receptor, e.g., an inhibiting receptor and an activating receptor. Bispecific antibodies
10 normally exhibit specific binding to two different antigens.

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variant refers to those nucleic acids that encode identical or essentially identical amino acid sequences. An example of a conservative substitution is the exchange of an amino acid in one of the
15 following groups for another amino acid of the same group (U.S. Pat. No. 5,767,063 issued to Lee, *et al.*; Kyte and Doolittle (1982) *J. Mol. Biol.* 157:105-132).

- (1) Hydrophobic: Norleucine, Ile, Val, Leu, Phe, Cys, Met;
- (2) Neutral hydrophilic: Cys, Ser, Thr;
- (3) Acidic: Asp, Glu;
- 20 (4) Basic: Asn, Gln, His, Lys, Arg;
- (5) Residues that influence chain orientation: Gly, Pro;
- (6) Aromatic: Trp, Tyr, Phe; and
- (7) Small amino acids: Gly, Ala, Ser.

25 Methods relating to polypeptide molecules having substantially the same amino acid sequence as CD200 or CD200R (SEQ ID NOs:2, 4, 6, 8, 10, 12) but possessing minor amino acid substitutions, truncations, or deletions, that do not substantially affect the functional aspects are within the definition of the contemplated invention. Variants containing one or more peptide bond cleavages, where daughter polypeptides remain in association with each
30 other, are within the definition of the contemplated invention.

"ITIM" and "ITAM" are two motifs found on some inhibiting and activating receptors, respectively. The ITIM motif is defined by the consensus sequence I/V/LxYxxL/V in the

cytoplasmic domain where (Y) can be phosphorylated, resulting in the ability of the polypeptide bearing the ITIM motif to recruit various enzymes, where the enzymes aid in relaying an inhibitory signal to the cell (Sathish, et al., (2001) *J. Immunol.* 166:1763-1770). The consensus ITAM sequence is YxxL/Ix₆₋₈YxxL/I, where (Y) may be phosphorylated
5 resulting in a change in signaling properties of the activating receptor or an accessory protein. The ITAM motif may occur within an activating receptor itself, or within an accessory protein that binds to the activating receptor, thus conferring activating properties to the activating receptor.

“Monofunctional reagent” refers, e.g., to an antibody, binding composition derived
10 from the binding site of an antibody, an antibody mimetic, a soluble receptor, engineered, recombinant, or chemically modified derivatives thereof, that specifically binds to a single type of target. For example, a monofunctional reagent may contain one or more functioning binding sites for an CD200 receptor. “Monofunctional reagent” also refers to a polypeptide, antibody, or other reagent that contains one or more functioning binding sites for, e.g., CD200 receptor
15 and one or more non-functioning binding sites for Fc receptor. For example, a monofunctional reagent may comprise an antibody binding site for CD200 receptor plus an Fc fragment that has been engineered so that the Fc fragment does not specifically bind to Fc receptor.

“Bifunctional reagent” refers, e.g., to an antibody, binding composition derived from the binding site of an antibody, an antibody mimetic, a soluble receptor, engineered,
20 recombinant, or chemically modified derivatives thereof, that specifically binds to two different targets, e.g., to an inhibiting CD200 receptor and an activating receptor. Generally, the bifunctional reagent will comprise binding sites from, e.g., two different antibodies, two different soluble receptors, or an antibody and a soluble receptor.

“Nucleic acid” refers to deoxyribonucleotides or ribonucleotides and polymers thereof
25 in either single stranded or double-stranded form. The term nucleic acid may be used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide. A particular nucleic acid sequence also implicitly encompasses “allelic variants” and “splice variants.”

“Specific binding” of a binding composition means that the binding composition binds to a specific antigen, e.g., CD200Ra (SEQ ID NO:2), with a binding constant ordinarily about
30 2-fold greater than to another antigen, typically about 4-fold greater than to another antigen, more typically at least about 10-fold greater than to another antigen, frequently at least about 40-fold greater than to another antigen; and most frequently at least about 100-fold greater than to another antigen.

“Ligand” refers to small molecules, peptides, polypeptides, and membrane associated or membrane-bound molecules that act as agonists or antagonists of a receptor, as well as to soluble versions of the above membrane-associated or membrane-bound molecules. Where the ligand is membrane-bound on a first cell, the receptor usually occurs on a second cell. The second cell may have the same or a different identity as the first cell. A ligand or receptor may be entirely intracellular, that is, it may reside in the cytosol, nucleus, or some other intracellular compartment. The ligand or receptor may change its location, e.g., from an intracellular compartment to the outer face of the plasma membrane. The complex of a ligand and receptor is termed a “ligand receptor complex.” Where a ligand and receptor are involved in a signaling pathway, the ligand occurs at an upstream position and the receptor occurs at a downstream position of the signaling pathway.

“Humanized antibody” means an antibody comprising an antigen-binding region of nonhuman origin, e.g., rodent, and at least a portion of an immunoglobulin of human origin, e.g., a human framework region, a human constant region, or portion thereof. See, e.g., U.S. Pat. No. 6,352,832.

“Immune condition” means, e.g., pathological inflammation, an inflammatory disorder, an inflammatory disease or disorder, or an autoimmune disorder or disease. “Immune condition” also refers to infections and cancerous conditions, e.g., pathological states where the immune system attempts to reduce the infection or reduce the cancerous condition.

“Cancerous condition” includes, e.g., cancer, cancer cells, tumors, angiogenesis, and precancerous conditions such as dysplasia.

“Sample” refers to a sample from a human, animal, or to a research sample, e.g., a cell, tissue, organ, fluid, gas, aerosol, slurry, colloid, or coagulated material. The “sample” may be tested in vivo, e.g., without removal from the human or animal, or it may be tested in vitro.

The sample may be tested after processing, e.g., by histological methods. “Sample” also refers, e.g., to a cell comprising a fluid or tissue sample or a cell separated from a fluid or tissue sample. “Sample” may also refer to a cell, tissue, organ, or fluid that is freshly taken from a human or animal, or to a cell, tissue, organ, or fluid that is processed or stored.

“Therapeutically effective amount” of a therapeutic agent is defined as an amount of each active component of the pharmaceutical formulation that is sufficient to show a meaningful patient benefit, i.e., to cause a decrease in or amelioration of the symptoms of the condition being treated. When the pharmaceutical formulation comprises a diagnostic agent, “a therapeutically effective amount” is defined as an amount that is sufficient to produce a signal,

image, or other diagnostic parameter. Effective amounts of the pharmaceutical formulation will vary according to factors such as the degree of susceptibility of the individual, the age, gender, and weight of the individual, and idiosyncratic responses of the individual. See, e.g., U.S. Pat. No. 5,888,530.

5

II. General.

The invention provides methods for the treatment and diagnosis of immune conditions, inflammatory conditions, and autoimmune disorders involving cells bearing CD200R, e.g., mast cells, antigen presenting cells (APCs), dendritic cells, neutrophils, T cells, monocytes, and
10 macrophages. Dendritic cells are professional APCs. These conditions include, e.g., rheumatoid arthritis, bronchial hyperreactivity, asthma, allergic conditions, psoriasis, inflammatory bowel disease, multiple sclerosis, and pathological innate response, e.g., endotoxemia, sepsis, and septic shock. The invention contemplates methods of modulating a CD200R using, e.g., an agonist or an antagonist of CD200Ra or CD200Rb.

15 The invention contemplates use of binding compositions to CD200Ra, e.g., for inhibiting mast cells in the treatment of mast cell-dependent pathological conditions. Mast cells are implicated in the initiation and prolongation of rheumatoid arthritis (RA) (Lee, et al. (2002) Science 297:1689-1692; Vastag (2002) J. Am. Med. Assoc. 288:1457-1458; Woolley and Tetlow (2000) Arthritis Res. 2:65-74; Olsson, et al. (2001) Ann. Rheum. Dis. 60:187-193).
20 Collagen-induced arthritis (CIA) is an experimental animal model for RA. RA and CIA involve, e.g., fibrin deposition, hyperplasia of synovial cells, periosteal bone formation, mononuclear infiltrates, pannus formation, and ankylosis of the joints (Luross and Willians (2001) Immunology 103:407-416). Immune cells, such as T cells and B cells, infiltrate the joints and induce pathology, e.g., inflammation, edema, or destruction of bone and cartilage.
25 RA is, in part, an autoimmune disorder, wherein autoimmunity occurs against various proteins, including cartilage structural proteins (Griffiths and Remmers (2001) Immunol. Revs. 184:172-183). CIA encompasses many features common to several human autoimmune diseases, e.g., RA, diabetes, multiple sclerosis, and autoimmune thyroiditis (Griffiths and Remmers, supra).

Mast cells also contribute to endotoxemia, which can be induced by administration of
30 LPS to mice and related conditions (Tuncel, et al. (2000) Peptides 21:81-89; Muchamuel, et al. (1997) J. Immunol. 158:2898-2903; Howard, et al. (1993) J. Exp. Med. 177:1205-1208). Endotoxemia correlates with sepsis, septic shock, infection with Gram negative and other

bacteria, and adverse reactions in innate immunity (Cohen (2000) Intensive Care Med., 26 Suppl. 1: S51-56; Freise, et al. (2001) J. Invest. Surg. 14:195-212).

Mast cells and APCs have been implicated in the pathology of skin disorders, e.g., psoriasis and atopic dermatitis. Psoriasis occurs in over 4% of the population of Western countries. Psoriasis, which may be life threatening in some instances, is characterized by frequent relapses. It has also been associated with a form of arthritis known as psoriatic arthritis (Ackermann and Harvima (1998) Arch. Dermatol. Res. 290:353-359; Yamamoto, et al. (2000) J. Dermatol. Sci. 24:171-176; Ackerman, et al. (1999) Br. J. Dermatol. 140:624-633; Schopf (2002) Curr. Opin. Invest. Drugs 3:720-724; Granstein (1996) J. Clin. Invest. 98:1695-1696; Christophers (2001) Clin. Exp. Dermatol. 26:314-320; Greaves and Weinstein (1995) New Engl. J. Med. 332:581-588; Robert and Kupper (1999) New Engl. J. Med. 341:1817-1828; Fearon and Veale (2001) Clin. Exp. Dermatol. 26:333-337; Mrowietz, et al. (2001) Exp. Dermatol. 10:238-245; Ackermann, et al. (1999) Br. J. Dermatol. 140:624-633).

Asthma is another disorder involving mast cells, APCs, and other immune cells (Black (2002) New Engl. J. Med. 346:1742-1743; Brightling, et al. (2002) New Engl. J. Med. 346:1699-1705; Carroll, et al. (2002) Eur. Respir. J. 19:1-7; Xiang and Nilsson (2000) Clin. Exp. Allergy 30:1379-1386; Woodruff and Fahy (2001) J. Am. Med. Assoc. 286:395-398). Asthma is a chronic disorder characterized by bronchial hyperreactivity, which is the manifestation of pulmonary inflammatory disorders, including asthma, chronic obstructive pulmonary disease (a.k.a. COPD; chronic obstructive pulmonary disorder), chronic bronchitis, eosinophilic bronchitis, bronchiolitis, and viral bronchiolitis (Riffo-Vasquez and Spina (2002) Pharmacol. Therapeutics 94:185-211). Asthma is the result of a cascade of immune events, including the release of IgE. (See, e.g., Marone (1998) Immunol. Today 19:5-9; Barnes and Lemanske (2001) New Engl. J. Med. 344:350-362).

Mast cells contribute to the pathology of inflammatory bowel disease, e.g., Crohn's disease and colitis (Raithel, et al. (2001) Scand. J. Gastroenterol. 36:174-179; Nishida, et al. (2002) Hepatogastroenterol. 49:678-682; Gelbmann, et al. (1999) Gut 45:210-217; Nolte, et al. (1990) Gut 31:791-794; Jeziorska, et al. (2001) J. Pathol. 194:484-492). IgE activates mast cells resulting in constriction of the airways and damage by eosinophils to the airways.

Mast cells also contribute to the pathology of inflammatory conditions of the nervous system, e.g., multiple sclerosis (Robbie-Ryan, et al. (2003) J. Immunol. 170:1630-1634; Dines and Powell (1997) J. Neuropathol. Exp. Neurol. 56:627-640). These cells also play a role in allograft rejection, e.g., of the liver, kidney, and lung and graft versus host disease (GVHD),

and glomerulonephritis (O'Keefe, *et al.* (2002) Liver Transpl. 8:50-57; Lajoie, *et al.* (1996) Mod. Pathol. 9:1118-1125; Yousem (1997) Hum. Pathol. 28:179-182; Levi-Schaffer and Weg (1997) Clin. Exp. Allergy, 27 Suppl. 1:64-70; Hiromura, *et al.* (1998) Am. J. Kidney Dis. 32:593-599). Mast cells have also been implicated in cardiovascular disease (Hara, *et al.* (2002) J. Exp. Med. 195:375-381).

In addition to mast cells, APCs have also been implicated in the mechanisms of disorders, such as, rheumatoid arthritis, allergies, asthma, endotoxemia, septic shock, and skin conditions, e.g., psoriasis (Santiago-Schwarz, *et al.* (2001) J. Immunol. 167:1758-1768; Lambrecht and Hammad (2003) Curr. Opin. Pulm. Med. 9:34-41; Eigenmann (2002) Pediatr. Allergy Immunol. 13:162-167; Curry, *et al.* (2003) Arch. Pathol. Lab. Med. 127:178-186; Supajatura, *et al.* (2002) J. Clin. Invest. 109:1351-1359; Koga, *et al.* (2002) Dermatol. 204:100-103).

The invention also contemplates a method of modulating a CD200R using, e.g., an antagonist of CD200Ra or an agonist of CD200Rb, in the treatment of infections or proliferative conditions, such as cancer and tumors. Mast cells, APCs, and other cells of the immune system play a role in preventing or combatting infections, e.g., bacterial, viral, and protozoal infections. See, e.g., Marshall, *et al.* (2003) Curr. Pharm. Dis. 9:11-24; Malaviya and Georges (2002) Clin. Rev. Allergy Immunol. 22:189-204; Mekori and Metcalfe (2000) Immunol. Rev. 173:131-140; Galli, *et al.* (1999) Curr. Opinion Immunol. 11:53-59; Miles and Mamlok (1992) J. Allergy Clin. Immunol. 89:638-639; Sacks and Sher (2002) Nature Immunol. 3:1041-1047; Eigenmann (2002) Pediatr. Allergy Immunol. 13:162-171). Mast cells, APCs, or other cells of the immune system have also been found to participate in preventing or combatting proliferative conditions, e.g., cancer and tumors. See, e.g., Reay (2001) Expert Opin. Ther. Targets 5:491-506; Heckelsmiller, *et al.* (2002) Eur. J. Immunol. 32:3235-3245; Stift, *et al.* (2003) Int. J. Oncol. 22:651-656; Vermorken and Van Tendeloo (2003) Expert Rev. Anticancer Ther. 3:1-3.

III. CD200 receptors.

Murine CD200Ra (a.k.a. muCD200Ra; SEQ ID NO:6) has a relatively long cytoplasmic tail. From *in vivo* studies, muCD200Ra is believed to be an inhibitory receptor, although it lacks a classical ITIM sequence. MuCD200Rb (SEQ ID NO:8), muCD200Rc (SEQ ID NO:10) and muCD200Rd (SEQ ID NO:12) have short cytoplasmic tails, charged amino acids in their transmembrane regions, and have been shown to pair with a cellular activating

adaptor molecule, Dap12 (Lanier and Bakker (2000) Immunol. Today 21:611-614). Human CD200Ra is homologous to murine CD200Ra. Human CD200Rb (SEQ ID NO:4) is most homologous to muCD200Rb/d and is also a pairing partner with Dap12.

5 IV. Purification and modification of polypeptides.

Polypeptides, e.g., antigens, antibodies, and antibody fragments, for use in the contemplated method can be purified by methods that are established in the art. Purification may involve homogenization of cells or tissues, immunoprecipitation, and chromatography. Stability during purification or storage can be enhanced, e.g., by anti-protease agents, anti-oxidants, ionic and non-ionic detergents, and solvents, such as glycerol or dimethylsulfoxide.

Modification to proteins and peptides include epitope tags, fluorescent or radioactive groups, monosaccharides or oligosaccharides, sulfate or phosphate groups, C-terminal amides, acetylated and esterified N-groups, acylation, e.g., fatty acid, intrachain cleaved peptide bonds, and deamidation products (Johnson, et al. (1989) J. Biol. Chem. 264:14262-14271; Young, et al. (2001) J. Biol. Chem. 276:37161-37165). Glycosylation depends upon the nature of the recombinant host organism employed or physiological state (Jefferis (2001) BioPharm 14:19-27; Mimura, et al. (2001) J. Biol. Chem. 276:45539-45547; Axford (1999) Biochim. Biophys. Acta 1:219-229; Malhotra, et al. (1995) Nature Medicine 1:237-243).

Derivatives of polypeptides also include modification by a fusion protein partner (Ausubel, et al. (2001) Current Protocols in Molecular Biology, Vol. 3, John Wiley and Sons, Inc., NY, NY, pp. 16.0.5-16.22.17; Sigma-Aldrich, Co. (2001) Products for Life Science Research, St. Louis, MO; pp. 45-89; Amersham Pharmacia Biotech (2001) BioDirectory, Piscataway, N.J., pp. 384-391).

25 V. Binding compositions.

Antibodies can be derived from human or non-human sources. Intact protein, denatured protein, or a peptide fragment of the protein, may be used for immunization (Harlow and Lane, supra, pp. 139-243). Regions of increased antigenicity may be used for peptide fragment immunization. Human CD200Ra (SEQ ID NO:2) has regions of increased antigenicity, e.g., at amino acids 43-47, 62-66, 109-114, 165-174, 187-199, 210-214, 239-244, 260-267, and 293-300 of SEQ ID NO:2 (Vector NTI® Suite, InforMax, Inc., Bethesda, MD). Human CD200Rb (SEQ ID NO:4) is unusually antigenic at amino acids 55-75 of SEQ ID NO:4. Mouse CD200Ra (SEQ ID NO:6) has regions of increased antigenicity at amino acids

25-40 and amino acids 85-95 of SEQ ID NO:6. Mouse CD200Rb (SEQ ID NO:8) has regions of increased antigenicity at amino acids 10-22, 85-90, and 105-120 of SEQ ID NO:8. Mouse CD200Rc (SEQ ID NO:10) has regions of increased antigenicity at amino acids 20-40, 115-130, and 190-220 of SEQ ID NO:10. Mouse CD200Rd has regions of increased antigenicity at amino acids 20-50, 90-120, 155-175, and 180-200 of SEQ ID NO:12 (MacVector 6.5®, Accelrys, San Diego, CA). This list of antigenic fragments and regions is not intended to limit the regions of the polypeptides that can be used to raise antibodies or that can be bound by antibodies.

Binding compositions comprising an extracellular domain of CD200, or antigenic fragments thereof, are contemplated, e.g., in mono- and bifunctional agents. The extracellular domain of human, mouse, and rat CD200 is described (Chen, *et al.* (1997) Biochim. Biophys. Acta 1362:6-10).

Antibodies derived from murine or other non-human sources can provoke an immune response, provide weak recruitment of effector function, or show rapid clearance from the bloodstream (Baca, *et al.* (1997) J. Biol. Chem. 272:10678-10684). For these reasons, it may be desired to prepare therapeutic antibodies by humanization. A humanized antibody contains the amino acid sequences from six complementarity determining regions (CDRs) of the parent mouse antibody, that are grafted on a human antibody framework. The content of non-human sequence in humanized antibodies is preferably low, i.e., about 5% (Baca, *et al.* (1997) J. Biol. Chem. 272:10678-10684). To achieve optimal binding, the humanized antibody may need fine-tuning, by changing certain framework amino acids, usually involved in supporting the conformation of the CDRs, back to the corresponding amino acid found in the parent mouse antibody. The framework amino acids that are generally changed back to those of the parent are those involved in supporting the conformation of the CDR loops (Chothia, *et al.* (1989) Nature 342:877-883; Foote and Winter (1992) J. Mol. Biol. 224:487-499). The framework residues that most often influence antigen binding is relatively small, and may be as small as eleven residues (Baca, *et al.* (1997) J. Biol. Chem. 272:10678-10684).

Humanized antibodies include antibodies having all types of constant regions, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. When it is desired that the humanized antibody exhibit cytotoxic activity, the constant domain is usually a complement-fixing constant domain and the class is typically IgG1. When such cytotoxic activity is not desirable, the constant domain can be of the IgG2 class. The humanized

antibody may comprise sequences from more than one class or isotype (U.S. Pat. No. 6,329,511 issued to Vasquez, et al.).

An alternative to humanization is to use human antibody libraries displayed on phage or human antibody libraries in transgenic mice (Vaughan, et al. (1996) Nature Biotechnol. 14:309-314; Barbas (1995) Nature Medicine 1:837-839; Mendez, et al. (1997) Nature Genetics 15:146-156; Hoogenboom and Chames (2000) Immunol. Today 21:371-377; Barbas, et al. (2001) Phage Display: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Kay, et al. (1996) Phage Display of Peptides and Proteins: A Laboratory Manual, Academic Press, San Diego, CA; de Bruin, et al. (1999) Nature Biotechnol. 17:397-399).

Bifunctional antibodies are provided. See, e.g., Mack, et al. (1995) Proc. Natl. Acad. Sci. USA 92:7021-7025; Carter (2001) J. Immunol. Methods 248:7-15; Volkel, et al. (2001) Protein Engineering 14:815-823; Segal, et al. (2001) J. Immunol. Methods 248:1-6; Brennan, et al. (1985) Science 229:81; Raso, et al. (1997) J. Biol. Chem. 272:27623; Morrison (1985) Science 229:1202; Traunecker, et al. (1991) EMBO J. 10:3655; and U.S. Pat. Nos. 5,932,448, 5,532,210, and 6,129,914. Single chain antibodies and diabodies are described. See, e.g., Malecki, et al. (2002) Proc. Natl. Acad. Sci. USA 99:213-218; Conrath, et al. (2001) J. Biol. Chem. 276:7346-7350; Desmyter, et al. (2001) J. Biol. Chem. 276:26285-26290; Hudson and Kortt (1999) J. Immunol. Methods 231:177-189; and U.S. Pat. No. 4,946,778.

Purification of antigen is not necessary for the generation of antibodies. Animals can be immunized with cells bearing the antigen of interest. Splenocytes can then be isolated from the immunized animals, and the splenocytes can fused with a myeloma cell line to produce a hybridoma (Meygaard, et al. (1997) Immunity 7:283-290; Wright, et al. (2000) Immunity 13:233-242; Preston, et al., supra; Kaithamana, et al. (1999) J. Immunol. 163:5157-5164).

Therapeutic antibodies may be conjugated, e.g., to small drug molecules, enzymes, liposomes, polyethylene glycol (PEG), or fusion protein antibodies. See, e.g., van Oosterhout, et al. (2001) Int. J. Pharm. 221:175-186; Marsh and Klinman (1990) 144:1046-1051; Kreitman (2001) Curr. Pharm. Biotechnol. 2:313-325; Dinndorf, et al. (2001) J. Immunother. 24:511-516; Wahl, et al. (2001) Int. J. Cancer 93:540-600; Garber (2000) J. Nat. Cancer Instit. 92:1462-1464; Everts, et al. (2002) J. Immunol. 168:883-889; Chen, et al. (2001) Int. J. Cancer 94:850-858; Shaik, et al. (2001) J. Control. Release 76:285-295; Park, et al. (2001) J. Control. Release 74:95-113; Solorzano, et al. (1998) J. Appl. Physiol. 84:1119-1130; Rosenberg, et al. (2001) J. Appl. Physiol. 91:2213-2223; Bendele, et al. (2000) Arthritis Rheum. 43:2648-2659;

Trakas and Tzartos (2001) J. Neurochem. 120:42-49; Chapman, et al. (1999) Nature Biotechnol. 17:780-783; Gaidamakova, et al. (2001) J. Control. Release 74:341-347; Coiffier, et al. (2002) New Engl. J. Med. 346:235-242.

Antibodies are useful for diagnostic or kit purposes, and include antibodies coupled, e.g., to dyes, radioisotopes, enzymes, or metals, e.g., colloidal gold (Le Doussal, et al. (1991) J. Immunol. 146:169-175; Gibellini, et al. (1998) J. Immunol. 160:3891-3898; Hsing and Bishop (1999) J. Immunol. 162:2804-2811; Everts, et al. (2002) J. Immunol. 168:883-889).

VI. Inhibiting and activating receptors.

The invention provides methods for cross-linking two different receptors, e.g., an inhibiting receptor and an activating receptor, for modulating cell activity.

Examples of inhibiting receptors include, e.g., FcγRIIB, LAIR, FDF03, KIR, gp49B, ILT25, PIR-B, Ly49, CTLA-4, CD200Ra (SEQ ID NO:2), CD94/NKG2A, NKG2B-E, PECAM-1, CD5, CD22, CD72, PIR1, SIRPα, HM18, LRC, ILT, KIR, LIR, MIR, and MAFA. See, e.g., Long (1999) Ann. Rev. Immunol. 17:875-904; Lanier (1997) Immunity 6:371-378; Sinclair (1999) Scan. J. Immunol. 50:10-13; Pan, et al. (1999) Immunity 11:495-506. Inhibiting receptors also include DNAX Surface Protein-1 (a.k.a. DSP-1) (Cantoni, et al. (1999) Eur. J. Immunol. 29:3148-3159).

Activating receptors include, e.g., CD3, CD2, CD10, CD161, Dap12, KAR, KARAP, FcεRI, FcεRII, FcγRIIA, FcγRIIC, FcγRIII/CD16, Trem-1, Trem-2, CD28, p44, p46, B cell receptor, LMP2A, STAM, STAM-2, GPVI, and CD40. See, e.g., Azzoni, et al. (1998) J. Immunol. 161:3493-3500; Kita, et al. (1999) J. Immunol. 162:6901-6911; Merchant, et al. (2000) J. Virol. 74:9115-9124; Pandey, et al. (2000) J. Biol. Chem. 275:38633-38639; Zheng, et al. (2001) J. Biol. Chem. 276:12999-13006; Propst, et al. (2000) J. Immunol. 165:2214-2221.

The invention provides methods for inhibiting, e.g., lymphoid cells, myeloid cells, and endothelial cells. Cell inhibition is accomplished, e.g., by treating a cell, tissue, organ, extracellular fluid, animal, human subject, or cells or tissues ex vivo, with a mono-, bi-, or multifunctional reagent or binding composition.

Agents that modulate expression of receptors, e.g., on a cell surface, are described. See, e.g., van de Winkel, et al. (1991) J. Leukocyte Biol. 49:511-524; van de Winkel, et al. (1993) Immunol. Today 14:215-221; Heijnen, et al. (1997) Intern. Rev. Immunol. 16:29-55; Fridman and Sautes (1996) Cell-Mediated Effects of Immunoglobins, Chapman and Hall, New York, NY, pp. 39-40). The invention encompasses using an agent to increase expression of an

inhibiting receptor, such as CD200Ra (SEQ ID NO:2), in order to increase efficiency of interaction of a binding composition specific for CD200Ra with CD200Ra, e.g., associated with mast cells, APCs, neutrophils, T cells, B cells, basophils, eosinophils, or epithelial cells. Also contemplated is use of an agent to increase expression of an activating receptor, such as CD200Rb (SEQ ID NO:4). The agent may comprise, e.g., a cytokine such as interferon or IL-10, a growth factor, a bifunctional reagent, an enzyme, or a small molecule such as adenosine. The agent may comprise a factor that promotes maturation of, e.g., mast cells, dendritic cells, or other APCs, or neutrophils, such as, stem cell factor, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor, or IL-6 (Hjertson, *et al.* (1999) Brit. J. Haematol. 104:516-522; Austen and Boyce (2001) Leuk. Res. 25:511-518; Vandenabeele and Wu (1999) Immunol. Cell Biol. 77:411-419; Santiago-Schwarz (1999) J. Leuk. Biol. 66:209-216; Liu, *et al.* (2001) Nat. Immunol. 2:585-589; Kondo, *et al.* (2003) Ann. Rev. Immunol.; Dumortier, *et al.* (2003) Blood 101:2219-2226).

VII. Screening.

Assays comprising animals, cells, or reagents such as beads or wells, are contemplated for screening for CD200, CD200R, and for agents that modulate interactions between CD200 and CD200R. See, e.g., Steinitz (2000) Analyt. Biochem. 232:238; Gast, *et al.* (1999) Analyt. Biochem. 276:227-241; Kaiser, *et al.* (2000) Analyt. Biochem. 282:173-185; and U.S. Pat. Nos. 6,176,962 and 6,517,234.

Cells or animals can be engineered, e.g., to express a CD200R, in order to facilitate their use in screening. Expression can be measured by, e.g., by hybridization-based techniques (Ausubel, *et al.* (2001) Curr. Protocols Mol. Biol., Vol. 4, John Wiley and Sons, New York, NY, pp. 25.0.1-25B.2.20; Ausubel, *et al.* (2001) Curr. Protocols Mol. Biol., Vol. 3, John Wiley and Sons, New York, NY, pp. 14.0.1-14.14.8; Liu, *et al.* (2002) Analyt. Biochem. 300:40-45; Huang, *et al.* (2000) Cancer Res. 60:6868-6874; Wittwer, *et al.* (1997) Biotechniques 22:130-138; Schmittgen, *et al.* (2000) Analyt. Biochem. 285:194-204; Heid, *et al.* (1996) Genome Res. 6:989-994). Polypeptides can be detected, e.g., by antibody-based techniques. See, e.g., Harlow and Lane, *supra*, pp. 553-612; Sims, *et al.* (2000) Analyt. Biochem. 281:230-232.

VIII. Therapeutic compositions.

Formulations of, e.g., binding compositions, binding compounds, or antibodies, are prepared by mixing, e.g., the antibody having the desired degree of purity with optional

physiologically acceptable carriers, excipients, or stabilizers in the form of lyophilized cake or aqueous solutions. See, e.g., Hardman, et al. (2001) Goodman and Gilman's the Pharmacological Basis of Therapeutics, McGraw-Hill, New York, NY; and Gennaro (2000) Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, NY; Avis, et al. (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY; and Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY. The therapeutic or pharmaceutical of this invention may be combined with or used in association with, e.g., anti-inflammatory, chemotherapeutic or chemopreventive agents.

Acceptable carriers, excipients, buffers, stabilizers, and surfactants are described. See, e.g., U.S. Pat. Nos. 6,342,220; 5,440,021; 6,096,728; and Weiner and Kotkoskie (2000) Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, NY.

Therapeutic antibody compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle. The route of antibody administration is, e.g. injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intracerebrospinal, intralesional, or pulmonary routes, or by sustained release systems. Sustained release systems are described. See, e.g.; Sidman et al. (1983) Biopolymers, 22:547-556; Langer et al. (1981) J. Biomed. Mater. Res. 15:167-277; Langer (1982) Chem. Tech. 12:98-105; Epstein et al. (1985) Proc. Natl. Acad. Sci. USA 82:3688-3692; Hwang et al. (1980) Proc. Natl. Acad. Sci. USA 77:4030-4034; U.S. Pat. Nos. 6,350,466 and 6,316,024.

An "effective amount" of antibody to be employed therapeutically will depend, e.g., upon the therapeutic objectives, the route of administration, the type of antibody employed, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the antibody until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays.

In the treatment or prevention of, e.g., an inflammatory or proliferative disorder, by the contemplated method, the binding composition will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical

condition of the individual patient, the cause of the disorder, the site of delivery of the antibody, the particular type of antibody, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of antibody to be administered will be governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat the proliferative disorder. Such amount is preferably below the amount that is toxic to the host.

As a general proposition, the initial pharmaceutically effective amount of the antibody administered parenterally will be in the range of about 0.1 $\mu\text{g/kg}$ to 10 mg/kg of the patient's body weight per day, ordinarily 0.1 $\mu\text{g/kg/day}$ to 1.0 mg/kg/day , preferably 0.1 $\mu\text{g/kg/day}$ to 0.1 mg/kg/day , more preferably 0.1 $\mu\text{g/kg/day}$ to 0.01 mg/kg/day , and most preferably 0.1 $\mu\text{g/kg/day}$, or less. The desired dosage can be delivered by a single bolus administration, by multiple bolus administrations, or by continuous infusion administration of antibody, depending on the pattern of pharmacokinetics that the practitioner wishes to achieve. As noted above, however, these suggested amounts of antibody are subject to a fair amount of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained.

IX. Secondary therapeutics.

The invention contemplates use of combinations of an agonist or antagonist of CD200R with a second agent, e.g., an anti-inflammatory, immunosuppressive, or anti-neoplastic agent. Anti-inflammatory agents include, e.g., steroids and non-steroidal anti-inflammatories (U.S. Pat. No. 6,294,170 issued to Boone, *et al.*; U.S. Pat. No. 6,096,728 issued to Collins, *et al.*; Hardman, *et al.* (2001) Goodman and Gilman's the Pharmacological Basis of Therapeutics, McGraw-Hill, New York, NY). Immunosuppressive agents include, e.g., azothioprine, mercaptopurine, and methotrexate. Anti-neoplastic agents include, e.g., 5-fluorouracil, methotrexate, cis-platin, and doxorubicin (U.S. Pat. No. 6,066,668 issued Hausheer, *et al.*).

X. Kits.

The contemplated method is adapted for use in kits, i.e., for screening or diagnostic purposes. The kit may be adapted for use in detecting or quantitating a binding composition to CD200R, e.g., in a pharmaceutical composition or in a biological sample. The contemplated kit is adapted for use with, e.g., mast cells from a subject or patient. Typically, the kit will have a compartment, a reagent, or directions for use or disposal, or any combination thereof.

Kits adapted to binding assays are described, e.g., U.S. Pat. Nos. 6,306,608; 6,150,122; 6,083,760; and 5,863,739.

XI. Uses.

5 The present invention contemplates methods for using a binding composition specific for a CD200R to modulate disorders associated with improper function of cells of the immune system, e.g., mast cells, APCs, such as dendritic cells, T cells, neutrophils, monocytes, or macrophages. The binding composition may comprise an agonist or antagonist of a CD200R. Agonists of an inhibiting receptor, e.g., CD200Ra (SEQ ID NOs:2 or 6), will be useful in
10 inhibiting activity of mast cells, APCs, such as dendritic cells, T cells, neutrophils, monocytes, or macrophages. Antagonists of an activating receptor, e.g., CD200Rb (SEQ ID NOs:4, 6, 10, or 12) will also be useful in inhibiting activity of, e.g., mast cells, APCs, such as dendritic cells, T cells, neutrophils, monocytes, or macrophages. Antagonists of CD200Ra and agonists of CD200Rb will be useful for stimulating, e.g., mast cells and APCs, such as dendritic cells, T
15 cells, neutrophils, monocytes, or macrophages, for the treatment of infections and proliferative conditions.

 The methods of the present invention contemplate use of, e.g., peptides, small molecules, antibodies, and binding compositions derived from the antigen binding site of an antibody, to treat or diagnose a variety of immune disorders, e.g., bronchial hyperreactivity,
20 allergic rhinitis, asthma, bronchitis, and anaphylaxis. See, e.g., Woodruff and Fahy, supra; Plaut (2001) J. Am. Med. Assoc. 286:3005-3006; and Marshall and Bienenstock (1994) Curr. Op. Immunol. 6:853-859; Luskin and Luskin (1996) Am. J. Ther. 3:515-520.

 The methods of the present invention can also be used to treat or diagnose rheumatoid arthritis, and skin disorders including psoriasis and atopic dermatitis. See, e.g., Mican and
25 Metcalf (1990) J. Allergy Clin. Immunol. 86:677-683; Malone, et al. (1987) Arthritis Rheum. 30:130-137; Malfait, et al. (1999) J. Immunol. 162:6278-6280; Ackermann and Harvima (1998) Arch. Dermatol. Res. 290:353-359; Ackermann, et al. (1999) Brit. J. Dermatol. 140:624-633; Askenase, et al. (1983) J. Immunol. 131:2687-2694. The method also contemplates treatment or diagnosis of inflammatory diseases of the gastrointestinal tract, e.g.,
30 Crohn's disease, ulcerative colitis, and irritable bowel syndrome. See, e.g., Malaviya, et al. (1995) Am. J. Ther. 2:787-792; Jeziorska, et al. (2001) J. Pathol. 194:484-492; Sullivan, et al. (2000) Neurogastroenterol. Motility 12:449; Nolte, et al. (1990) Gut 31:791-794; Raithel, et al. (2001) Scand. J. Gastroenterol. 36:174-179. The method is also contemplated for use in

treating chronic liver disease and congestive heart failure (O'Keefe, *et al.* (2002) Liver Transpl. 8:50-57; Hara, *et al.* (2002) J. Exp. Med. 195:375-381).

The invention also encompasses methods of treatment or diagnosis of demyelination or neurodegeneration, e.g., multiple sclerosis, Guillain-Barre syndrome, Alzheimer's disease, Parkinson's disease, and epilepsy (Purcell and Atterwill (1995) Neurochem. Res. 20:521-532; Secor, *et al.* (2000) J. Exp. Med. 191:813-822; Dines and Powell (1997) J. Neuropathol. Exp. Neurol. 56:627-640; Purcell and Atterwill (1995) Neurochem. Res. 20:521-532; Dietsch and Hinrichs (1989) J. Immunol. 142:1476-1481).

Moreover, the invention encompasses a method of treating or diagnosing Sjogren's syndrome, transplant and graft rejection, graft-versus-host disease (GVHD), mastocytosis, and methods for preventing angiogenesis (Pedersen and Nauntofte (2001) Expert Opin. Pharmacother. 2:1415-1436; Kontinen, *et al.* (2000) Rheumatol. Int. 19:141-147; Moutsopoulos and Youinou (1991) Curr. Opin. Rheumatol. 3:815-822, Gorczynski, *et al.* (2000) Clin. Immunol. 95:182-189; Koskinen, *et al.* (2001) Transplantation 71:174-1747; O'Keefe, *et al.* (2002) Liver Transpl. 8:50-57; Lajoie, *et al.* (1996) Mod. Pathol. 9:1118-1125; Pardo, *et al.* (2000) Virchows Arch. 437:167-172; Yousem (1997) Hum. Pathol. 28:179-182; and Levi-Schaffer and Wej (1997) Clin. Exp. Allergy 27 Suppl. 1:64-70; Tomita, *et al.* (2000) Ann. Thorac. Surg. 69:1686-1690; Brockow and Metcalfe (2001) Curr. Opin. Allergy Clin. Immunol. 1:449-454; Hiromatsu and Toda (2003) Microsc. Res. Tech. 60:64-69).

Yet another aspect of the invention provides methods of modulating activity of a CD200R, e.g., SEQ ID NO:2, for the treatment or prevention of cardiovascular disease, e.g., atherosclerosis. Immune cells, e.g., mast cells, dendritic cells, neutrophils, monocytes, and macrophages, contribute to the pathology of atherosclerosis. See, e.g., Huang, *et al.* (2002) Cardiovasc. Res. 55:150-160; Kelley, *et al.* (2000) Mol. Med. Today 6:304-308; Aicher, *et al.* (2003) Circulation 107:604-611; Ozmen, *et al.* (2002) Histol. Histopathol. 17:223-237; Wanders, *et al.* (1994) Transpl. Int. 7 Suppl. 1:S371-S375.

Also encompassed are methods of providing an antagonist of CD200Ra (SEQ ID NO:2) or an agonist of CD200Rb (SEQ ID NO:4) to stimulate cell activity, e.g., to combat bacterial infections, viral infections, persistent infections, infections by foreign multi-cellular organisms, cancerous conditions and tumors, and to promote wound healing.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments.

EXAMPLES

I. General Methods.

5 Methods for the diagnosis and treatment of inflammatory conditions in animals and in humans are described. See, e.g., Ackerman (1997) Histological Diagnosis of Inflammatory Skin Disease, 2nd ed., Lippincott, Williams, and Wilkins, New York, NY; Gallin, et al. (1999) Inflammation: Basic Principles and Clinical Correlates, 3rd ed., Lippincott, Williams, and Wilkins, New York, NY; Geppetti and Holzer (1996) Neurogenic Inflammation, CRC Press, 10 Boca Raton, FL; Nelson, et al. (2000) Cytokines in Pulmonary Disease: Infection and Inflammation, Marcel Dekker, Inc., New York, NY; O'Byrne (1990) Asthma as an Inflammatory Disease, Marcel Dekker, Inc., New York, NY., Parnham, et al. (1991) Drugs in Inflammation (Agents and Actions Suppl., Vol. 32), Springer Verlag, Inc., New York, NY; Benezra (1999) Ocular Inflammation: Basic and Clinical Concepts, Blackwell Science, Ltd., 15 Oxford, UK.

 Standard methods in molecular biology are described (Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Sambrook and Russell (2001) Molecular Cloning, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Wu (1993) Recombinant DNA, Vol. 217, Academic Press, 20 San Diego, CA). Standard methods also appear in Ausbel, et al. (2001) Current Protocols in Molecular Biology, Vols. 1-4, John Wiley and Sons, Inc. New York, NY, which describes cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4).

 Methods for protein purification including immunoprecipitation, chromatography, 25 electrophoresis, centrifugation, and crystallization are described (Coligan, et al. (2000) Current Protocols in Protein Science, Vol. 1, John Wiley and Sons, Inc., New York). Chemical analysis, chemical modification, post-translational modification, and glycosylation of proteins are described (Coligan, et al. (2000) Current Protocols in Protein Science, Vol. 2, John Wiley and Sons, Inc., New York). Production, purification, and fragmentation of polyclonal and 30 monoclonal antibodies is described (Coligan, et al. (2001) Current Protocols in Immunology, Vol. 1, John Wiley and Sons, Inc., New York; Harlow and Lane (1999) Using Antibodies, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Harlow and Lane, supra).

Standard techniques for characterizing ligand/receptor interactions are available. See, e.g., Coligan, et al. (2001) Current Protocols in Immunology, Vol. 4, John Wiley and Sons, Inc., New York.

Standard techniques in cell and tissue culture are described. See, e.g., Freshney (2000) Culture of Animal Cells: A Manual of Basic Technique, 4th ed., Wiley-Liss, Hoboken, NJ; Masters (ed.) (2000) Animal Cell Culture: A Practical Approach, 3rd ed., Oxford Univ. Press, Oxford, UK; Doyle, et al. (eds.) (1994) Cell and Tissue Culture: Laboratory Procedures, John Wiley and Sons, NY; Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY.

Animal models for arthritis, multiple sclerosis, psoriasis, and lipopolysaccharide (LPS)-induced inflammation are available. See, e.g., Luross and Williams (2001) Immunology 103:407-416; Griffiths and Remmers (2001) Immunol. Rev. 184:172-183; Beurler (2000) Curr. Opin. Immunol. 12:20-26; Campbell, et al. (1998) J. Immunol. 161:3639-3644; Tompkins, et al. (2002) J. Immunol. 168:4173-4183; Hong, et al. (1999) J. Immunol. 162:7480-7491.

Software packages for determining, e.g., antigenic fragments, signal and leader sequences, protein folding, and functional domains, are available. See, e.g., Vector NTI® Suite (Informax, Inc., Bethesda, MD); GCG Wisconsin Package (Accelrys, Inc., San Diego, CA), and DeCypher® (TimeLogic Corp., Crystal Bay, Nevada); Menne, et al. (2000) Bioinformatics 16:741-742. Public sequence databases were also used, e.g., from GenBank and others.

II. Preparation of mast cells.

Murine mast cell cultures were established from 2-3 week old C57BL/6 mice. Bone marrow was flushed from the femurs of 2-3 mice and subsequently washed three times with phosphate buffered saline (PBS). Cells were resuspended in 15 ml Dulbecco's minimal essential media (MEM) supplemented with sodium pyruvate, non-essential amino acids, 2-mercaptoethanol, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), glutamine, 10-15% fetal calf serum (Hyclone, Inc., Logan, UT), 100 ng/mg rSCF, and 100 ng/ml of rIL-3. Cells were incubated in T25 flasks at 37°C. At weekly intervals, the non-adherent cells were refed with fresh media and transferred to a new flask. At two weeks, culture media was supplemented with 5.0 ng/ml of IL-4. By four weeks, the majority of non-adherent cells are

expected to be typical murine mast cells expressing IgE FcR. From four weeks on, the cells were maintained with supplements of only rIL-3 and rIL-4.

III. Distribution of CD200R.

5 Total RNA was isolated from a variety of cell types and tissues using the RNeasy® RNA isolation kit (Qiagen, Inc., Valencia, CA). RNA was reverse transcribed using oligo dT primers and Multiscribe® reverse transcriptase (Applied Biosystems, Inc., Foster City, CA). cDNA was analyzed for expression of CD200 receptors and ubiquitin by Taqman® PCR assays using a Perkin Elmer ABI Prism 5700® sequence detection system (Perkin Elmer, Inc.,
10 Wellesley, MA). Quantitation of target gene expression was calculated by normalizing the values relative to the expression of ubiquitin.

Results of Taqman® analysis are presented in Table 1. Highest expression is represented by (+++), moderate expression is (++) or (+), while borderline to undetectable expression levels is (-). ND means not determined.

Table 1.

Cell or tissue	Human CD200Ra (SEQ ID NOs:1,2)	Human CD200Rb (SEQ ID NO:3,4)	Mouse CD200Ra (SEQ ID NO:5,6)	Mouse CD200Rb (SEQ ID NO:7,8)	Mouse CD200Rc (SEQ ID NO:9,10)	Mouse CD200Rd (SEQ ID NO:11,12)
Mast cells	(++)	(+++)	(++)	(+++)	(+++)	(+++)
Macrophages resting ex bone marrow	ND	ND	(+++)	(+++)	(-)	(++)
Macrophages activated LPS + IFNg + IL-10 24 hour ex bone marrow	ND	ND	(++)	(++)	(-)	(++)
Brain Macrophages (microglia)	ND	ND	(+)	(+)	(-)	(+)
Activated microglia	ND	ND	(+)	(++)	(-)	(++)
Monocytes	(+)	(+,-)	ND	ND	ND	ND
Dendritic cells	(+++)	(+++)	(+)	(++)	(+)	(+)
B cells	(+,-)	(+)	(+,-)	(+,-)	(-)	(+,-)
T cell C57BL/6 TH1 activated pool	ND	ND	(+,-)	(+,-)	(-)	(-)
T cell C57BL/6 TH2 activated pool	ND	ND	(++)	(++)	(+,-)	(+)
T cell TH1 activated pool	(-)	(-)	ND	ND	ND	ND
T cell TH2 activated pool	(+)	(-)	ND	ND	ND	ND
NK cell	(-)	(-)	ND	ND	ND	ND
Endothelial cell	ND	ND	(-)	(+)	(-)	(-)
Fibroblast	(-)	(+,-)	(-)	(-)	(-)	(-)
Aorta atherosclerosis model	ND	ND	(+++)	(+++)	(-)	(+++)

Table 1 (continued).

Cell or tissue	Human CD200Ra (SEQ ID NO:1,2)	Human CD200Rb (SEQ ID NO:3,4)	Mouse CD200Ra (SEQ ID NO:5,6)	Mouse CD200Rb (SEQ ID NO:7,8)	Mouse CD200Rc (SEQ ID NO:9,10)	Mouse CD200Rd (SEQ ID NO:11,12)
Colon	(++)	(-)	(+)	(++)	(+,-)	(+)
Lung	(++)	(++)	(+++)	(++)	(++)	(++)
Lung hypersensitivity pneumonitis	(+++)	ND	ND	ND	ND	ND
Fibroblast hypersensitivity pneumonitis	(+++)	ND	ND	ND	ND	ND
Eosinophilic granuloma	(++)	ND	ND	ND	ND	ND
Lung idiopathic pulmonary fibrosis	(++)	ND	ND	ND	ND	ND
Infiltrating cells rheumatoid arthritis	(+++)	ND	ND	ND	ND	ND
Skin	(-)	(-)	(++)	(+)	(+)	(+)
Spinal cord C57BL/6 TNF α knockout, untreated	ND	ND	(+,-)	(+,-)	(-)	(-)
Spinal cord C57BL/6 TNF α knockout EAE model	ND	ND	(+)	(+)	(-)	(++)
Spleen	ND	ND	(++)	(+)	(++)	(+)

IV. Association of CD200R with Dap12.

5 To verify the association of CD200 receptors with Dap12, CD200 receptors were introduced by retrovirus vectors into Baf cell lines that had previously been transfected with one of the following FLAG-tagged molecules: Dap12, Dap10, Fc ϵ R γ , or CD3- ζ . The FLAG-tagged molecules are only expressed on the cell surface when stably associated with appropriate pairing partner molecule. When murine CD200Rc (SEQ ID NO:10) or murine

10 CD200Rd (SEQ ID NO:12) was introduced into these transfectants, only FLAG-tagged Dap12 was rescued, indicating that these CD200 receptors specifically pair with Dap12.

V. Agonist antibodies of CD200R.

Epitope-tagged CD200R molecules were stably introduced into normal mouse mast cells. These CD200Rs were then directly engaged with monoclonal antibodies specific for various epitope tags. Mast cell degranulation, cytokine secretion, and proliferation were monitored with and without subsequent cross-linking of the anti-tag antibodies. Triggering CD200Ra had no effects on resting mast cells, however, triggering the CD200 receptors that pair with Dap12, i.e., activating CD200Rs, caused significant mast cell degranulation, cytokine secretion, and autocrine-dependent proliferation.

Epitope-tagged CD200Ra was stably introduced into normal mast cells. CD200Ra (SEQ ID NO:6) further comprising an epitope tag was engaged by a CD200 Ig-fusion protein or by monoclonal antibodies against the epitope tags while simultaneously activating the FcεRI with IgE antibodies. Mast cells were then monitored for degranulation, cytokine secretion, and proliferative responses. In some experiments, CD200Ra and FcεRI were co-ligated by a secondary antibody. The results demonstrated that CD200Ra is an inhibitory receptor capable of inhibiting FcεRI-dependent responses in mast cells.

In related studies, monoclonal antibodies specific for murine CD200Ra (SEQ ID NO:6) were used in place of the anti-tag antibodies. In these studies with murine mast cells, the results again demonstrated that CD200Ra is an inhibitory receptor.

Monoclonal antibodies specific for human CD200Ra were tested for agonist activity using activated mast cells. Anti-huCD200Ra antibodies, i.e., DX136 (rIgG2a) and DX139 (rIgM), were found to be agonists of CD200Ra as measured by assessing changes in degranulation and secretion, using murine mast cells expressing human CD200Ra. Agonist activity is expressed by IC50.

Fusion proteins, comprising two CD200 extracellular domains fused to an Fc region, were also tested for agonist activity (huCD200-Ig; muCD200-Ig). These fusion proteins contained a mutation (D265A in the constant regions of the Fc used to create both huCD200-Ig and muCD200-Ig) to prevent binding to Fc receptor (FcR) and to complement (Idusogie, *et al.* (2000) *J. Immunol.* 164:4178-4184). These fusion proteins also delivered a signal that inhibited mast cell degranulation and cytokine secretion.

FACS analysis of blood cells revealed the types of white blood cells expressing CD200R, where cells were tagged by the indicated antibody (Table 2). Receptor internalization was measured by confocal microscopy (Liu, *et al.* (1998) *Cancer Res.* 58:4055-

4060; Lee, et al. (2000) J. Pharmacol. Exp. Therapeutics 292:1048-1052) (Table 2). ND means not determined. Immunohistochemistry (IHC) refers to the ability of the indicated mAb to stain cells in frozen, acetone-fixed, human or mouse tissue, or formalin-fixed, paraffin-embedded human or mouse tissues.

Table 2.

Agonists of human CD200Ra (SEQ ID NO:2)						
Name	Species and isotype	Agonist activity	Bioassay IC50	FACS analysis of blood	IHC	Receptor internalization
DX136	Rat IgG2a	Yes	1.0 nM	T cells+ Monocytes+ Neutrophils+++ Basophils++	Yes	No
DX138	Rat IgG2a	Yes	40 nM	T cells+ Monocytes+ Neutrophils+++ Basophils++	Yes	Yes
DX139	Rat IgM	Strong	0.01 nM	T cells+ Neutrophils+++ Basophils+	Yes	No
DX142	Rat IgG1	Yes	0.5 nM	Neutrophils+	No	ND
DX147	Rat IgG1	Yes	0.2 nM	Neutrophils+	Yes	No
DX153	Rat IgG1	Yes	0.14 nM	T cells(+,-); monocytes (+); neutrophils (+++); basophils (+)		
huCD200-Ig	Mouse IgG1	Yes	0.3 nM	ND	ND	ND
Agonists of mouse CD200Ra (SEQ ID NO:6)						
Name	Species and isotype	Agonist activity	Bioassay IC50	FACS analysis of blood	IHC	Receptor internalization
DX109	Rat IgG1	yes	0.2 nM	T cells+ Monocytes++ Neutrophils+++	Yes	No
DX110	Rat IgG1	yes, less than DX109	ND	ND	Yes	ND
muCD200-Ig	Mouse IgG1	yes	1.3 nM	ND	ND	ND

Mouse mast cells expressing the human CD200Ra molecule were stimulated with 25 $\mu\text{g/ml}$ monoclonal antibody to trigger the Dap12-linked activating receptor of mouse CD200Rd, followed by measurement of secreted β -hexosaminidase at 1.0 hours (Table 2).

5 Agonist activity of anti-CD200Ra antibody or CD200-Ig fusion protein was assessed by adding anti-CD200Ra antibody or CD200-Ig fusion protein to the cell incubation mixtures and determining inhibition of secretion. Incubations were conducted with antibodies or CD200-Ig fusion protein at concentrations of 0, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, and 9.0 $\mu\text{g/ml}$. Control cell incubations were conducted with the rat isotype control mAb.

10 The results demonstrated that treatment with each of the anti-CD200R antibodies and with the CD200-Ig fusion protein inhibited mast cell degranulation. Control isotype antibodies did not detectably influence secretion. However, each of the tested compounds inhibited with differing efficiencies, as determined by the IC_{50} (Table 2). In addition to having a better agonistic effect, the antibodies' effect was also longer lasting than that of the Ig-fusion protein.

15 Mouse mast cells were stimulated with IgE anti-TNP with antigen (TNP-keyhole limpet hemocyanin; KLH), followed by assay of released β -hexosaminidase (degranulation; Abs₄₀₅₋₆₅₀) and cytokine (secretion) (Figs. 1-2). The degranulation signal, IgE anti-TNP, was used at the indicated concentrations. DX109 monoclonal anti-CD200R antibody (mAb) and control rat IgG1 mAb were tested for their effect on inhibiting mast cell activity. DX109 mAb or rat IgG1 mAb were added at a constant concentration of 13 nM. The cytokines assayed were IL-13 and tumor necrosis factor (TNF) (Figs. 1-2). Degranulation, as assessed by hexosaminidase release, was studied (Fig. 1). Increases in stimulant concentration were followed by increases in hexosaminidase release where control rat IgG1 was added (upper curve, closed squares, Fig. 1), while incubations containing added DX109 mAb had relatively low levels of
20 hexosaminidase release, except at the highest level of stimulant (lower curve, closed triangles, Fig. 1). Increasing stimulant concentration resulted in increases in secretion of IL-13 (closed circles, Fig. 2) and TNF (closed squares, Fig. 2) where secretion of both cytokines was inhibited by DX109 monoclonal antibody. The results demonstrate that anti-CD200R antibody is effective in inhibiting degranulation and cytokine secretion.

30 VI. Cross-linking an inhibiting receptor with an activating receptor.

Degranulation and secretion by human mast cells was measured by a protocol involving addition of anti-IgE receptor antibody, which binds to IgE receptor, addition of anti-CD200R

antibody, which binds to CD200Ra (SEQ ID NO:2), and addition of goat anti-mouse F(ab')₂, which binds simultaneously to the anti-IgE antibody (adhering to IgE receptor) and to anti-CD200R antibody (adhering to CD200R). Control experiments involved variations of this protocol.

5 Whole cord blood cells were cultured in Yssels medium supplemented with stem cell factor (SCF) and IL-6 for 4-6 weeks to produce human mast cells. IL-4 and IgE were added to the culture for an additional 2 weeks. Cells were then plated at 10⁶ cells/well in 96 well flat bottom plates. An inhibitory antibody (anti-CD200Ra antibody) or control antibody (mouse Ig) was then added. After 20 min incubation, anti-IgE receptor antibody was added to a
10 concentration of 20 ng/ml. After 20 min of further incubation, the wells were washed and the crosslinker (goat anti-mouse antibody) was added. The mixture was incubated for 1 h, and the supernatant withdrawn and used for degranulation assays, as assessed by tryptase release. Tryptase assays were performed with the substrate N-alpha-benzyl-DL-arginine p-nitroanilide hydrochloride (BAPNA) with color measurement at 405-570 nm.

15 Degranulation (tryptase release) was maximal with addition of anti-IgE receptor antibody and control antibody (mouse Ig). Maximal tryptase release, under these conditions, resulted in Abs.₄₀₅₋₅₇₀ = 0.44-0.51. In incubations with anti-CD200Ra antibody, titrating levels of anti-CD200Ra antibody were used (0-1000 ng/ml anti-CD200Ra antibody). Different levels of antibody were used in separate incubation mixtures. Anti-CD200Ra antibody at increasing
20 levels resulted in the progressive inhibition of tryptase release, where maximal inhibition (Abs.₄₀₅₋₅₇₀ = 0.05) occurred with about 1000 ng/ml anti-CD200Ra antibody. Intermediate levels of anti-CD200Ra antibody (200 ng/ml) inhibited tryptase release to about 25% the level of maximal tryptase release. The results demonstrate that cross-linking CD200Ra with IgE receptor prevents IgE receptor-dependent degranulation in human mast cells.

25 Cross-linking of various inhibitory receptors, other than CD200Ra (SEQ ID NO:2), with FcεRI also inhibited mast cell activity. The inhibitory receptors studied were DSP-1 and LAIR-1. Anti-DSP-1 antibody or anti-LAIR-1 antibody were used as agonists for DSP-1 or LAIR-1, respectively. Degranulation (tryptase release) was maximal with addition of anti-IgE receptor antibody plus control antibody (mouse Ig). Maximal tryptase release, under these
30 conditions, resulted in Abs.₄₀₅₋₅₇₀ = 0.44-0.51. Titrating levels of anti-DSP-1 antibody were used (0-1000 ng/ml anti-DSP-1 antibody), with different levels of anti-DSP-1 antibody in separate incubation mixtures. Increasing concentrations of anti-DSP-1 antibody resulted in progressive inhibition of tryptase release, where maximal inhibition (Abs₄₀₅₋₅₇₀ = 0.08)

occurred at about 40 ng/ml anti-DSP-1 antibody, as well as at higher concentrations of anti-DSP-1 antibody. Intermediate concentrations of anti-DSP-1 antibody (about 8 ng/ml) resulted in 25% maximal tryptase release. The results demonstrate that cross-linking DSP-1 with IgE receptor prevents IgE receptor-dependent degranulation.

5 In studies involving the inhibitory receptor LAIR-1, anti-LAIR-1 antibody was used at 0 or 50 ng/ml. Where incubations contained only activating antibody (anti-IgE receptor antibody), tryptase release was about $Abs_{405-570} = 0.69$ (defined as maximal). Where incubations contained activating antibody (anti-IgE receptor), anti-LAIR-1 antibody (50 ng/ml), and cross-linker, tryptase release was inhibited, and where tryptase release at the inhibited level
10 was about 10% maximal ($Abs_{405-570} = 0.07$). Control incubations containing no activating antibody resulted in very little tryptase release ($Abs_{405-570} = 0.06$). The results demonstrate that cross-linking LAIR-1 with IgE receptor prevents IgE receptor-dependent degranulation.

VII. Preventing mast cell degranulation in vivo.

15 To determine if agonism of the CD200Ra molecule could also prevent mast cell degranulation in vivo, the passive cutaneous anaphylaxis (PCA) was employed. Each mouse in a group of five 30 g CD1 mice was injected i.v. with 100ug of DX109 (rat anti-muCD200Ra mAb). Each mouse in a second group of five, 30g CD1 mice was injected i.v. with 100 µg of rat IgG1 control mAb. One hour later, the backs of all ten mice were shaved and mouse IgE
20 anti-DNP mAb was injected intradermally (i.d.) in a volume of 20 µl at 3 sites per mouse, respectively 10, 20 or 40 ng IgE per site. Twenty four hours later, all mice received i.v. a mixture of antigen (DNP-HSA) and the dye Evans's blue. Where the injected antigen binds to, and cross-links the IgE bound to skin mast cells, degranulation occurs, leading to release of
25 mediators such as histamine that cause vascular edema, enabling the movement of the blue dye from the blood into the skin and appearance of blue spots on the skin. The size of the blue spot is proportional to the amount of IgE injected into the skin at that site. In mice receiving 100 µg of the control IgG1 mAb, the PCA reaction proceeded and blue spots appeared in all five mice, the largest size spot at the site where 40ng IgE was injected, the smallest blue spot where 10 ng IgE was injected. In mice receiving the DX109 anti-CD200Ra mAb, no blue spots appeared at
30 any concentration of injected IgE. Thus, as in the in vitro studies, the DX109 mAb delivered an inhibitory signal to the skin mast cells in vivo, preventing degranulation.

VIII. Treatment of collagen induced arthritis (CIA) with anti-CD200Ra antibody.

CIA was induced in mice followed by treatment with anti-CD200Ra antibody (DX109 mAb) or with control IgG antibody. Female B10.RIII mice, 8-10 weeks old, were treated with bovine type II collagen in complete Freund's adjuvant (CFA) (Jirholt, *et al.* (1998) *Eur. J. Immunol.* 28:3321-3328). At day 0, mice were treated with one dose of bovine Type II collagen in CFA. Starting at day 7, antibody was administered at weekly intervals. DX109 antibody and control IgG doses were 1 mg, i.p. at days 7 and 14, and 1 mg, s.c. at days 21 and 28. Disease scoring commenced at day 14 and was continued to day 45. Experimental mice received DX109, while control mice received control IgG.

CIA was scored by cumulative incidence in units of percent. The control group showed 0% score from t = 14-18 days; 40% score at t = 19-20 days; 60% score at t = 21-30 days; and 80% score at t = 31-44 days. The DX109-treated group showed 0% score from t = 14-24 days and 20% score from t = 25-44 days. Thus DX109-treatment resulted in lower scores and relief from CIA.

CIA was also scored by Mean Clinical Score (MCS) (Hoek, *et al.*, *supra*). The control group showed a MCS = 0 at 14-19 days; MCS = 1 at 20-21 days; MCS = ~2 at 22-31 days, followed by a progressive increase to MCS = ~4.5, where a score of MCS = ~4.5 occurred at days 39-44. The DX109-treated group showed MCS = 0 at 14-24 days, MCS = 0.2 at 25-26 days, MCS = 0.5 at 27-34 days, MCS = 0.2 at 35-42 days, and MCS = 0.5 at 43-44 days. Again, the results demonstrated that DX109-treatment resulted in lower scores and relief from CIA.

IX. Effects of anti-CD200R antibody on LPS-induced endotoxaemia.

Endotoxaemia was induced in mice by LPS-treatment followed by treatment with anti-CD200R antibody (DX109) or control antibody and assessment of survival rate. The LPS dose was adjusted to provide sufficient toxicity to provoke toxicity and death of the mice, while avoiding saturating levels that would prevent the antibody from enhancing survival. The mice were of the C57BL/6 strain. Mice received 0.5 mg antibody (i.p.) at two time points, i.e., 1 hour prior to as well as simultaneously with the LPS. The anti-CD200R antibody treated mice showed a higher survival rate at various time points when compared to the control group. The results demonstrated that anti-CD200R antibody protects mice from LPS-induced toxicity.

X. Distribution of CD200Ra in human skin.

CD200Ra was visualized in green by rat anti-CD200Ra and tagging with goat anti-rat containing Alexa Fluor®-488 (Molecular Probes, Eugene, OR). Mast cells were visualized in red with mouse anti-CD117 (a marker for mast cells) and tagging with goat anti-mouse containing Alexa Fluor®-594 (Molecular Probes, Eugene, OR). Staining with anti-CD117 revealed an array of discrete red spots. Staining with both antibodies indicated that about one third of CD200Ra-bearing cells were mast cells.

Normal human skin was also tested for localization of CD200Ra (SEQ ID NO:2) on macrophages. CD200Ra was localized by staining with anti-CD200Ra tagged with green.

Macrophages were located with anti-CD11b antibody tagged with red. Staining with either antibody alone or with both antibodies together demonstrated that a majority of macrophages expressed CD200Ra.

Normal human and psoriatic skin was probed with anti-CD200Ra (DX136), stained with horse radish peroxidase (HRP), and examined under visible light. The results showed that psoriatic skin was profusely stained in both the dermis and epidermis, when compared to normal skin. The psoriatic skin contained large clusters of CD200Ra⁺ cells, where these cells appeared to consist of T cells and myeloid cells.

SEQUENCE IDENTIFIERS

- SEQ ID NO:1 is human CD200Ra nucleic acid.
- 5 SEQ ID NO:2 is human CD200Ra polypeptide.
- SEQ ID NO:3 is human CD200Rb nucleic acid.
- SEQ ID NO:4 is human CD200Rb polypeptide.
- SEQ ID NO:5 is murine CD200Ra nucleic acid.
- SEQ ID NO:6 is murine CD200Ra polypeptide.
- 10 SEQ ID NO:7 is murine CD200Rb nucleic acid.
- SEQ ID NO:8 is murine CD200Rb polypeptide.
- SEQ ID NO:9 is murine CD200Rc nucleic acid.
- SEQ ID NO:10 is murine CD200Rc polypeptide.
- SEQ ID NO:11 is murine CD200Rd nucleic acid.
- 15 SEQ ID NO:12 is murine CD200Rd polypeptide.
- SEQ ID NO:13 is rat CD200R nucleic acid.
- SEQ ID NO:14 is rat CD200R polypeptide.

20 All citations herein are incorporated herein by reference to the same extent as if each individual publication, patent application, or patent was specifically and individually indicated to be incorporated by reference including all figures and drawings.

Many modifications and variations of this invention, as will be apparent to one of ordinary skill in the art, can be made to adapt to a particular situation, material, composition of matter, process, process step or steps, to preserve the objective, spirit, and scope of the invention. All such modifications are intended to be within the scope of the claims appended hereto without departing from the spirit and scope of the invention. The specific

25 embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of the equivalents to which such claims are entitled; and the invention is not to be limited by the specific

30 embodiments that have been presented herein by way of example.

WHAT IS CLAIMED IS:

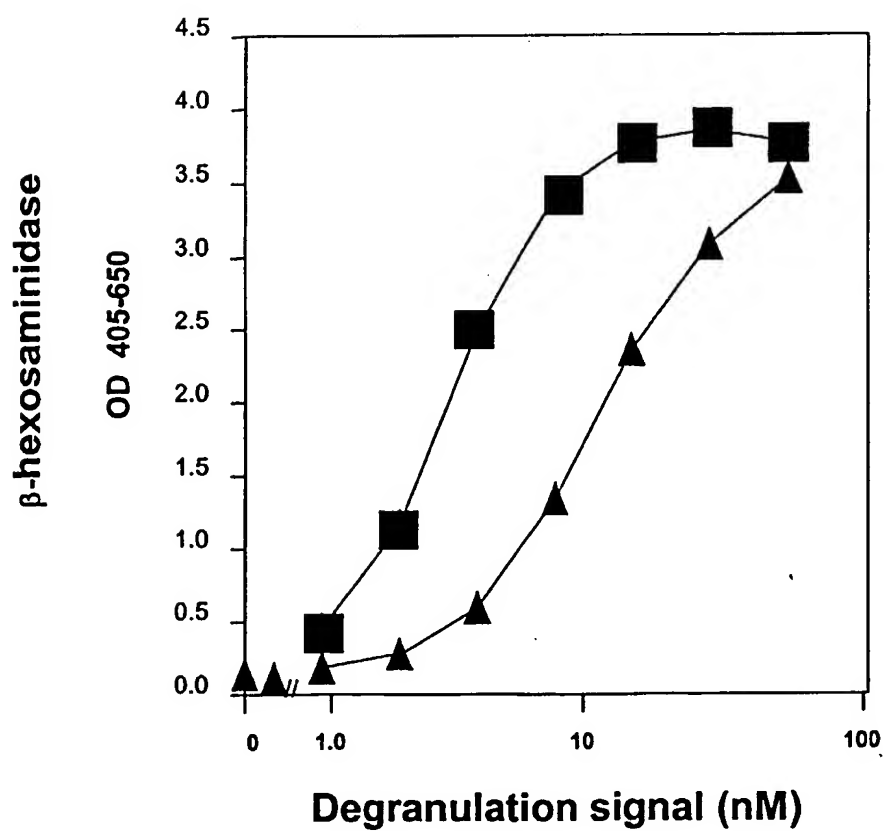
1. A method of modulating the activity of a cell comprising contacting the cell with a
5 binding composition derived from the antigen binding site of an antibody that specifically binds to CD200Ra (SEQ ID NOs:2 or 6), or an antigenic fragment thereof.
2. The method of Claim 1, wherein the cell is a mast cell.
- 10 3. The method of Claim 1, wherein the modulating:
 - a) inhibits cell activity; or
 - b) stimulates cell activity.
4. The method of Claim 1, wherein the modulating inhibits cell activity and the binding
15 composition comprises an agonist of CD200Ra (SEQ ID NOs:2 or 6).
5. The method of Claim 1, wherein the modulating increases cell activity and the binding composition comprises an antagonist of CD200Ra (SEQ ID NOs:2 or 6).
- 20 6. The method of Claim 1, wherein the binding composition comprises:
 - a) a humanized antibody;
 - b) a monoclonal antibody;
 - c) a polyclonal antibody;
 - d) an Fab fragment;
 - 25 e) an F(ab')₂ fragment;
 - f) a peptide mimetic of an antibody; or
 - g) a detectable label.
7. The method of Claim 1, further comprising contacting the cell with an agent that
30 enhances expression of CD200Ra (SEQ ID NOs:2 or 6), or an antigenic fragment thereof.
8. A method of treating a subject suffering from an immune condition comprising treating with or administering the binding composition of Claim 1.

9. The method of Claim 8, wherein the binding composition comprises an agonist or antagonist of CD200Ra (SEQ ID NOs:2 or 6).
- 5 10. The method of Claim 8, wherein the immune condition is:
a) an inflammatory condition; or
b) an autoimmune condition.
- 10 11. The method of Claim 8, wherein the immune condition is:
a) rheumatoid arthritis;
b) endotoxemia;
c) psoriasis; or
d) allergy.
- 15 12. The method of Claim 8, wherein the immune condition is:
a) an infection; or
b) a cancerous condition.
- 20 13. The method of Claim 8, wherein the binding composition is administered in conjunction with an agent that specifically enhances expression of CD200Ra (SEQ ID NOs:2 or 6), or an antigenic fragment thereof.
- 25 14. A method of diagnosing an immune disorder comprising contacting a sample with a binding composition of Claim 1 and determining the modulation of cell activity.
15. The method of Claim 14, wherein the modulation is:
a) inhibition; or
b) activation.
- 30 16. The method of Claim 14, wherein the contacting is in vitro.

METHODS OF MODULATING OX2 RECEPTORS

Cherwinski, et al.; DX01550K

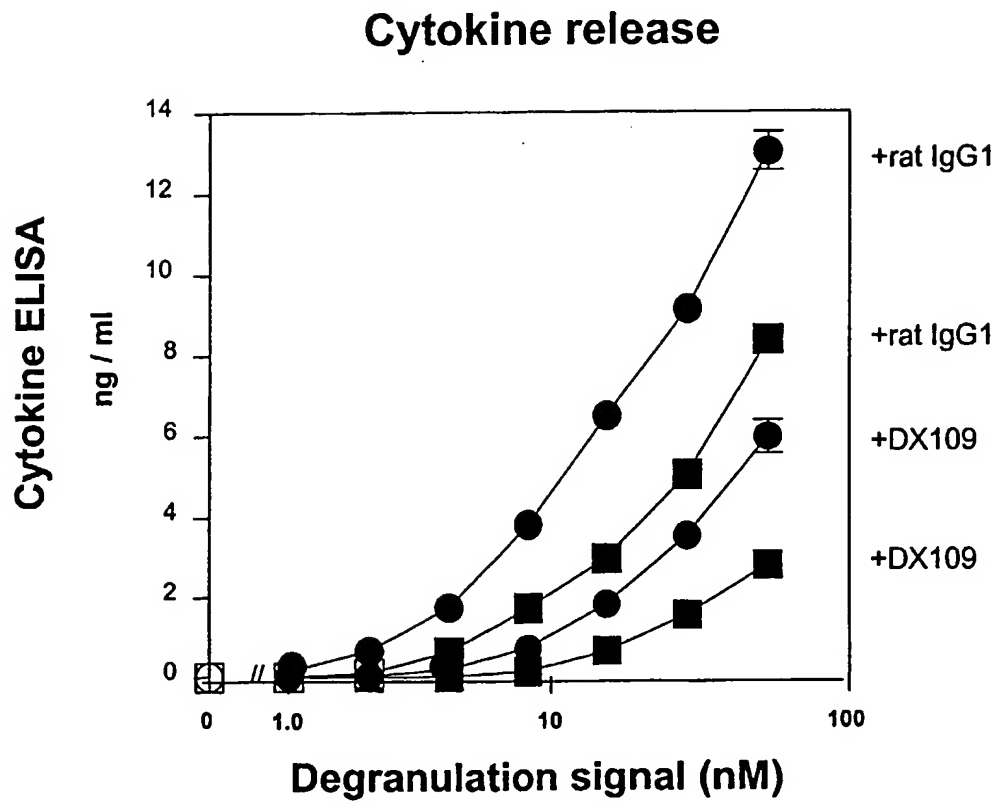
1/2

**Degranulation**

METHODS OF MODULATING OX2 RECEPTORS

Cherwinski, et al.; DX01550K

2/2



SEQUENCE LISTING

<110> Schering Corporation

<120> Methods of modulating CD200 receptors

<130> DX01550K WI

<150> 60/364,513

<151> 2002-03-15

<160> 14

<170> PatentIn version 3.1

<210> 1

<211> 2255

<212> DNA

<213> Homo sapiens

<400> 1

cagagaaaag cttctgttcg tccaagttac taaccaggct aaaccacata gacgtgaagg	60
aaggggctag aaggaaggga gtgcccact gttgatgggg taagaggatc ctgtactgag	120
aagttgacca gagagggtct caccatgcgc acagttcctt ctgtaccagt gtggaggaaa	180
agtactgagt gaagggcaga aaaagagaaa acagaaatgc tctgcccttg gagaactgct	240
aacctagggc tactgttgat ttgactatc ttcttagtgg ccgaagcgga ggggtgctgt	300
caaccaaaca actcattaat gctgcaaact agcaaggaga atcatgcttt agcttcaagc	360
agtttatgta tggatgaaaa acagattaca cagaactact cgaaagtact cgcagaagtt	420
aacacttcat ggcctgtaaa gatggctaca aatgctgtgc ttgttgccc tcctatcgca	480
ttaagaaatt tgatcataat aacatgggaa ataatcctga gaggccagcc ttcctgcaca	540
aaagcctaca ggaaagaaac aaatgagacc aaggaaacca actgtactga tgagagaata	600
acctgggtct ccagacctga tcagaattcg gaccttcaga ttcgtccagt ggccatcact	660
catgacgggt attacagatg cataatggta acacctgatg ggaatttcca tcgtggatat	720
cacctccaag tgtagttac acctgaagtg accctgtttc aaaacaggaa tagaactgca	780
gtatgcaagg cagttgcagg gaagccagct gcgcagatct cctggatccc agagggcgat	840
tgtgccacta agcaagaata ctggagcaat ggcacagtga ctgttaagag tacatgccac	900
tgggaggtcc acaatgtgtc taccgtgacc tgccacgtct cccatttgac tggcaacaag	960
agtctgtaca tagagctact tcctgttcca ggtgccaaaa aatcagcaaa attatatatt	1020
ccatatatca tccttactat tattattttg accatcgtgg gattcatttg gttgttgaaa	1080
gtcaatggct gcagaaaata taaattgaat aaaacagaat ctactccagt tgttgaggag	1140
gatgaaatgc agccctatgc cagctacaca gagaagaaca atcctctcta tgatactaca	1200


```

aacaaggtga aggcattctca ggcattacaa agtgaagttg acacagacct ccatacttta 1260
taagttgttg gactctagta ccaagaaaca acaacaaacg agatacatta taattactgt 1320
ctgattttct tacagttcta gaatgaagac ttatattgaa attaggtttt ccaaggttct 1380
tagaagacat tttaatggat tctcattcat acccttgat aattggaatt tttgattctt 1440
agctgctacc agctagttct ctgaagaact gatgttatta caaagaaaat acatgcccat 1500
gaccaaatat tcaaattgtg caggacagta aataatgaaa accaaatttc ctcaagaaat 1560
aactgaagaa ggagcaagtg tgaacagttt cttgtgtatc ctttcagaat attttaatgt 1620
acatatgaca tgtgtatatg cctatggat atgtgtcaat ttatgtgtcc ccttacatat 1680
acatgcacat atctttgtca aggcaccagt gggaacaata cactgcatta ctgttctata 1740
catatgaaaa cctaataata taagtcttag agatcatttt atatcatgac aagtagagct 1800
acctcattct ttttaatggt tatataaaat tccattgtat agttatatca ttatttaatt 1860
aaaaacaacc ctaatgatgg atatttagat tcttttaagt tttgtttatt tcttttaagt 1920
tttgtttgtg gtataaacia taccacatag aatgtttctt gtgcatatat ctctttgttt 1980
ttgagtatat ctgtaggata actttcttga gtggaattgt caggtcaaag ggtttgtgca 2040
ttttactatt gatatatatg ttaattgtg tcaaatatat atgtcaaatt ccctccaaca 2100
ttgtttaaat gtgcctttcc cttaaattct attttaataa ctgtactatt cctgcttcta 2160
cagttgccac tttctctttt taatcaacca gattaaatat gatgtgagat tataataaga 2220
attatactat ttaataaaaa tggatttata ttttt 2255

```

<210> 2
 <211> 348
 <212> PRT
 <213> Homo sapiens

<400> 2

```

Met Leu Cys Pro Trp Arg Thr Ala Asn Leu Gly Leu Leu Leu Ile Leu
1           5           10           15

```

```

Thr Ile Phe Leu Val Ala Glu Ala Glu Gly Ala Ala Gln Pro Asn Asn
          20           25           30

```

```

Ser Leu Met Leu Gln Thr Ser Lys Glu Asn His Ala Leu Ala Ser Ser
          35           40           45

```

```

Ser Leu Cys Met Asp Glu Lys Gln Ile Thr Gln Asn Tyr Ser Lys Val
          50           55           60

```

```

Leu Ala Glu Val Asn Thr Ser Trp Pro Val Lys Met Ala Thr Asn Ala

```

65		70		75		80									
Val	Leu	Cys	Cys	Pro	Pro	Ile	Ala	Leu	Arg	Asn	Leu	Ile	Ile	Ile	Thr
				85					90					95	
Trp	Glu	Ile	Ile	Leu	Arg	Gly	Gln	Pro	Ser	Cys	Thr	Lys	Ala	Tyr	Arg
			100					105					110		
Lys	Glu	Thr	Asn	Glu	Thr	Lys	Glu	Thr	Asn	Cys	Thr	Asp	Glu	Arg	Ile
		115					120					125			
Thr	Trp	Val	Ser	Arg	Pro	Asp	Gln	Asn	Ser	Asp	Leu	Gln	Ile	Arg	Pro
	130					135					140				
Val	Ala	Ile	Thr	His	Asp	Gly	Tyr	Tyr	Arg	Cys	Ile	Met	Val	Thr	Pro
145					150					155					160
Asp	Gly	Asn	Phe	His	Arg	Gly	Tyr	His	Leu	Gln	Val	Leu	Val	Thr	Pro
			165						170					175	
Glu	Val	Thr	Leu	Phe	Gln	Asn	Arg	Asn	Arg	Thr	Ala	Val	Cys	Lys	Ala
			180					185					190		
Val	Ala	Gly	Lys	Pro	Ala	Ala	Gln	Ile	Ser	Trp	Ile	Pro	Glu	Gly	Asp
		195					200					205			
Cys	Ala	Thr	Lys	Gln	Glu	Tyr	Trp	Ser	Asn	Gly	Thr	Val	Thr	Val	Lys
	210					215					220				
Ser	Thr	Cys	His	Trp	Glu	Val	His	Asn	Val	Ser	Thr	Val	Thr	Cys	His
225					230					235					240
Val	Ser	His	Leu	Thr	Gly	Asn	Lys	Ser	Leu	Tyr	Ile	Glu	Leu	Leu	Pro
			245					250						255	
Val	Pro	Gly	Ala	Lys	Lys	Ser	Ala	Lys	Leu	Tyr	Ile	Pro	Tyr	Ile	Ile
			260					265					270		
Leu	Thr	Ile	Ile	Ile	Leu	Thr	Ile	Val	Gly	Phe	Ile	Trp	Leu	Leu	Lys
		275					280					285			
Val	Asn	Gly	Cys	Arg	Lys	Tyr	Lys	Leu	Asn	Lys	Thr	Glu	Ser	Thr	Pro
	290					295					300				
Val	Val	Glu	Glu	Asp	Glu	Met	Gln	Pro	Tyr	Ala	Ser	Tyr	Thr	Glu	Lys
305					310					315					320

Asn Asn Pro Leu Tyr Asp Thr Thr Asn Lys Val Lys Ala Ser Gln Ala
 325 330 335

Leu Gln Ser Glu Val Asp Thr Asp Leu His Thr Leu
 340 345

<210> 3

<211> 1010

<212> DNA

<213> Homo sapiens

<400> 3

```

atgggtggaa agcagatgac acagaactat tcaacaattt ttgcagaagg taacatttca      60
cagcctgtac tgatggatat aaatgctgtg ctttgttgcc ctccatttgc attaagaaat      120
ttgatcataa taacatggga aataatcctg agaggccagc cttcctgcac aaaagcctac      180
aagaaagaaa caaatgagac caaggaaacc aactgtactg ttgagagaat aacctgggtc      240
tctagacctg atcagaattc ggaccttcag attcgtccgg tggacaccac tcatgacggg      300
tattacagag gcatagtggg aacacctgat gggaatttcc atcgtggata tcacctcaa      360
gtgttagtta caccgaagt gacacctattt caaagcagga atataactgc agtatgcaag      420
gcagttacag ggaagccagc tgcccagatc tcttgatcc cagagggatc tattcttgcc      480
actaagcaag aatactgggg caatggcaca gtgacggtta agagtacatg cccctgggag      540
ggccacaagt ctactgtgac ctgccatgtc tccatttga ctggcaacaa gagtctgtcc      600
gtaaagtga attcagggtc cagaacctca ggatctccag cgttgtcctt actgatcatt      660
ctttatgtga aactctctct ttttgtggtc attctgggtc ccacaggatt tgttttcttc      720
cagaggataa atcatgtcag aaaagttctt taaagaagaa ggaaggggtc tcttttgctt      780
ctctccttg tctctggact gcaacattgg tgagatgagt gatgggtccag cagtgaactt      840
gggccatgga tgatgttaag gatagaagcc actcagtagg atagaagaaa agaaagatgg      900
aagaaggatc ctgggcttga tgaccatgaa gtttcctat aaaccctcaa ccacctattc      960
attgacttct tttgtgttag agtgaataaa attttgttca tgccagtgtt      1010

```

<210> 4

<211> 250

<212> PRT

<213> Homo sapiens

<400> 4

Met Gly Gly Lys Gln Met Thr Gln Asn Tyr Ser Thr Ile Phe Ala Glu
 1 5 10 15

Gly Asn Ile Ser Gln Pro Val Leu Met Asp Ile Asn Ala Val Leu Cys
 20 25 30

Cys Pro Pro Ile Ala Leu Arg Asn Leu Ile Ile Ile Thr Trp Glu Ile
 35 40 45

Ile Leu Arg Gly Gln Pro Ser Cys Thr Lys Ala Tyr Lys Lys Glu Thr
 50 55 60

Asn Glu Thr Lys Glu Thr Asn Cys Thr Val Glu Arg Ile Thr Trp Val
 65 70 75 80

Ser Arg Pro Asp Gln Asn Ser Asp Leu Gln Ile Arg Pro Val Asp Thr
 85 90 95

Thr His Asp Gly Tyr Tyr Arg Gly Ile Val Val Thr Pro Asp Gly Asn
 100 105 110

Phe His Arg Gly Tyr His Leu Gln Val Leu Val Thr Pro Glu Val Asn
 115 120 125

Leu Phe Gln Ser Arg Asn Ile Thr Ala Val Cys Lys Ala Val Thr Gly
 130 135 140

Lys Pro Ala Ala Gln Ile Ser Trp Ile Pro Glu Gly Ser Ile Leu Ala
 145 150 155 160

Thr Lys Gln Glu Tyr Trp Gly Asn Gly Thr Val Thr Val Lys Ser Thr
 165 170 175

Cys Pro Trp Glu Gly His Lys Ser Thr Val Thr Cys His Val Ser His
 180 185 190

Leu Thr Gly Asn Lys Ser Leu Ser Val Lys Leu Asn Ser Gly Leu Arg
 195 200 205

Thr Ser Gly Ser Pro Ala Leu Ser Leu Leu Ile Ile Leu Tyr Val Lys
 210 215 220

Leu Ser Leu Phe Val Val Ile Leu Val Thr Thr Gly Phe Val Phe Phe
 225 230 235 240

Gln Arg Ile Asn His Val Arg Lys Val Leu
 245 250

<210> 5
 <211> 1624
 <212> DNA
 <213> Mus musculus

<400> 5
 aaaaccgaaa tgttttgctt ttggagaact tctgccctag cagtgtctctt aatatggggg 60
 gtctttgtgg ctgggtcaag ttgtactgat aagaatcaaa caacacagaa caacagttca 120
 tctcctctga cacaagtga cactacagtgt tctgtacaga taggtacaaa ggctctgtctc 180
 tgctgtctttt ctattccact gacaaaagca gtattaatca catggataat aaagctcaga 240
 ggctgtccat cctgcacaat agcatacaaa gtagatacaa agaccaatga aaccagctgc 300
 ttgggcagga acatcacctg ggctccaca cctgaccaca gtcctgaact tcagatcagt 360
 gcagtgaccc tccagcatga ggggacttac acatgtgaga cagtaacacc tgaagggaat 420
 tttgaaaaaa actatgacct ccaagtgtgt gtgccccctg aagtaacctt ctttccagag 480
 aaaaacagat ctgcagtctg tgaggcaatg gcaggcaagc ctgctgcaca gatctcttgg 540
 tctccagatg gggactgtgt cactacgagt gaatcacaca gcaatggcac tgtgactgtc 600
 aggagcacat gccactggga gcagaacaat gtgtctgatg tgtcctgcat tgtctctcat 660
 ttgactggta accaatctct tcccatagaa ctgagtagag gtggtaacca atcattacga 720
 ccatatattc catacatcat accatcaatt atcattttga tcatcatagg atgcatttgt 780
 cttttgaaaa tcagtggctt cagaaaatgc aaattgcaa aattagaagc tacttcagct 840
 attgaggagg atgaaatgca gccttatgct agctatacag agaagagcaa tccactctat 900
 gatactgtga ctaaggtgga ggcatttcca gtatcacaa gcgaagtcaa tggcacagac 960
 tgccttactt tgtcggccat tggaaatctag aaccaagaaa aaagaagtca agagacatca 1020
 taattactgc tttgctttct ttaaaattcg acaatggaag gactacttgg aaattagctc 1080
 ttccaaagct attaaaaagc acaaatgttc taatgaaatt gcatttaaatt tctatcattg 1140
 gaagtttgga atctctgctg ctacctgtta attttaggaa gaactgattt aattattaca 1200
 aagaaagcac atggttatgg tgaaatatca agttgtgcaa taaagtatga tgaaaactga 1260
 gtttcctcaa gaaataactg caggaggaac aatcatcact aaagaatttc atgtgagttc 1320
 ttacaaaaaa attcctatgt atacatgact atggtatgtg tgtccaatta catgtttatt 1380
 tacaatgtg tatatatgca cacatttgtt ttccaggaca tctccttgta aaaaacacac 1440
 tggagttttg gatattataa agcttataaa gtgagcattg gagatatttt tatatcagca 1500
 taagtaaate tacctcattc tttttaatgg ctacatagaa ttctcctgta tgattacatt 1560
 gtaatttaat taatcatggc ccttggaatt tgtgtgtgtg gtggtattaa aaaaaccat 1620
 gtag 1624

<210> 6
 <211> 326
 <212> PRT
 <213> Mus musculus

<400> 6

Met Phe Cys Phe Trp Arg Thr Ser Ala Leu Ala Val Leu Leu Ile Trp
 1 5 10 15

Gly Val Phe Val Ala Gly Ser Ser Cys Thr Asp Lys Asn Gln Thr Thr
 20 25 30

Gln Asn Asn Ser Ser Ser Pro Leu Thr Gln Val Asn Thr Thr Val Ser
 35 40 45

Val Gln Ile Gly Thr Lys Ala Leu Leu Cys Cys Phe Ser Ile Pro Leu
 50 55 60

Thr Lys Ala Val Leu Ile Thr Trp Ile Ile Lys Leu Arg Gly Leu Pro
 65 70 75 80

Ser Cys Thr Ile Ala Tyr Lys Val Asp Thr Lys Thr Asn Glu Thr Ser
 85 90 95

Cys Leu Gly Arg Asn Ile Thr Trp Ala Ser Thr Pro Asp His Ser Pro
 100 105 110

Glu Leu Gln Ile Ser Ala Val Thr Leu Gln His Glu Gly Thr Tyr Thr
 115 120 125

Cys Glu Thr Val Thr Pro Glu Gly Asn Phe Glu Lys Asn Tyr Asp Leu
 130 135 140

Gln Val Leu Val Pro Pro Glu Val Thr Tyr Phe Pro Glu Lys Asn Arg
 145 150 155 160

Ser Ala Val Cys Glu Ala Met Ala Gly Lys Pro Ala Ala Gln Ile Ser
 165 170 175

Trp Ser Pro Asp Gly Asp Cys Val Thr Thr Ser Glu Ser His Ser Asn
 180 185 190

Gly Thr Val Thr Val Arg Ser Thr Cys His Trp Glu Gln Asn Asn Val
 195 200 205

Ser Asp Val Ser Cys Ile Val Ser His Leu Thr Gly Asn Gln Ser Leu
210 215 220

Ser Ile Glu Leu Ser Arg Gly Gly Asn Gln Ser Leu Arg Pro Tyr Ile
225 230 235 240

Pro Tyr Ile Ile Pro Ser Ile Ile Ile Leu Ile Ile Ile Gly Cys Ile
245 250 255

Cys Leu Leu Lys Ile Ser Gly Phe Arg Lys Cys Lys Leu Pro Lys Leu
260 265 270

Glu Ala Thr Ser Ala Ile Glu Glu Asp Glu Met Gln Pro Tyr Ala Ser
275 280 285

Tyr Thr Glu Lys Ser Asn Pro Leu Tyr Asp Thr Val Thr Lys Val Glu
290 295 300

Ala Phe Pro Val Ser Gln Gly Glu Val Asn Gly Thr Asp Cys Leu Thr
305 310 315 320

Leu Ser Ala Ile Gly Ile
325

<210> 7

<211> 1386

<212> DNA

<213> Mus musculus

<400> 7

agaggccagc cttcctgcat aatggcctac aaagtagaaa caaaggagac caatgaaacc	60
tgcttgggca ggaacatcac ctgggcctcc acacctgacc acattcctga ccttcagatc	120
agtgcggtgg ccctccagca tgaggggaat tacttatgtg agataacaac acctgaaggg	180
aatttccata aagtctatga cctccaagtg ctggtgcccc ctgaagtaac ctactttctc	240
ggggaaaata gaactgcagt ttgtgaggca atggcaggca agcctgctgc acagatctct	300
tggactccag atggggactg tgtcactaag agtgagtcac acagcaatgg cactgtgact	360
gtcaggagca cttgccactg ggagcagaac aatgtgtctg ctgtgtcctg cattgtctct	420
cattcgactg gtaatcagtc tctgtccata gaactgagta gaggtaccac cagcaccacc	480
ccttccttgc tgaccattct ctacgtgaaa atggtccttt tggggattat tcttcttaaa	540
gtgggatttg ttttcttcca gaagagaaat gttaccagaa catgaatatc cagatttctg	600
gaagctcatt agtctgatga cacataccag aaaacagcat ttgtaatcaa ctttctcatt	660
ggaatccagc ttaccctgcc ctgctgtctt catgtttgtt agacactcac ctccaaattc	720

```

ttaactgaga agggctcctg tctaaaggaa atatggggac aaattgtgga gcatagacca      780
aaagaaaggc catccagaga ctgccccacc taaggaccca tcccatatac agacaccaaaa      840
cccagacact actgaagatg ctgcgaagcg tttgtgtgaca ggagcctgtt atagctgtct      900
cctgagaggc tcagccagag cctgacaaat acataggtag atgcttgacag ccaacaactg      960
gactgagcaa aaaatctcca ttggaggagt tagagaaagg actgaagagg gtgaaaggg      1020
ttgcagcccc ataggaagaa caacaatata aaccaaccag atctcccaga gctcccagg      1080
actaaattac caaccaaagg ctacacatgg aaggacctat ggctccagct gcttgtgtag      1140
cagtggatgg ccttgttggg catcagtggg aggagaaacc cttggtccag taaaggcttg      1200
attccctagt gtaagagaat gccagggcag tgacgtggga gtgagtaggt aggaagcatc      1260
ctcatagatg caggagaaaag gagaatggaa gaggggtattc tggaggggaa actggaaaag      1320
gagacaacat ttgaaatgta aatacataaa atatccaata aaaaatgtac agttgccagt      1380
catgtg                                     1386

```

```

<210> 8
<211> 194
<212> PRT
<213> Mus musculus

```

```

<400> 8

```

```

Arg Gly Gln Pro Ser Cys Ile Met Ala Tyr Lys Val Glu Thr Lys Glu
1           5           10           15

```

```

Thr Asn Glu Thr Cys Leu Gly Arg Asn Ile Thr Trp Ala Ser Thr Pro
20           25           30

```

```

Asp His Ile Pro Asp Leu Gln Ile Ser Ala Val Ala Leu Gln His Glu
35           40           45

```

```

Gly Asn Tyr Leu Cys Glu Ile Thr Thr Pro Glu Gly Asn Phe His Lys
50           55           60

```

```

Val Tyr Asp Leu Gln Val Leu Val Pro Pro Glu Val Thr Tyr Phe Leu
65           70           75           80

```

```

Gly Glu Asn Arg Thr Ala Val Cys Glu Ala Met Ala Gly Lys Pro Ala
85           90           95

```

```

Ala Gln Ile Ser Trp Thr Pro Asp Gly Asp Cys Val Thr Lys Ser Glu
100          105          110

```


Ser His Ser Asn Gly Thr Val Thr Val Arg Ser Thr Cys His Trp Glu
 115 120 125

Gln Asn Asn Val Ser Ala Val Ser Cys Ile Val Ser His Ser Thr Gly
 130 135 140

Asn Gln Ser Leu Ser Ile Glu Leu Ser Arg Gly Thr Thr Ser Thr Thr
 145 150 155 160

Pro Ser Leu Leu Thr Ile Leu Tyr Val Lys Met Val Leu Leu Gly Ile
 165 170 175

Ile Leu Leu Lys Val Gly Phe Ala Phe Phe Gln Lys Arg Asn Val Thr
 180 185 190

Arg Thr

<210> 9
 <211> 1354
 <212> DNA
 <213> Mus musculus

<400> 9
 ggcacgagtt acgatttgtg cttaacctga ctccactcca gatgcatgct ttggggagga 60
 ctctggcttt gatgttactc atcttcatca ctattttggg gcctgagtcā agttgttcag 120
 tgaaaggacg ggaggagatc ccaccggatg attcatttcc tttttcagat gataatatct 180
 tccctgatgg agtgggctgc accatggaga ttgagattat cactccagtg tctgtacaga 240
 taggtatcaa ggctcagctt ttctgtcatc ctagtccatc aaaagaagca acacttagaa 300
 tatgggaaat aactcccaga gactggcctt cctgcagact accctacaga gcagagttgc 360
 agcagatcag taaaaaaatc tgtactgaga gaggaaccac tagggtcctt gcacatcacc 420
 agagttctga ccttcccatc aaatcaatgg ccctcaagca tgatgggcat tactcatgtc 480
 ggatagaaac aacagatggg attttccaag agagacatag catccaagtg ccaggggaaa 540
 atagaactgt agtttgtgag gcaattgcaa gcaagcctgc tatgcagatc ttgtggactc 600
 cagatgagga ctgtgtcact aagagtaaāt cacacaatga caccatgatt gtcaggagca 660
 agtgccacag ggagaaaaac aatggccaca gtgtgttctg ctttatctcc catttgactg 720
 ataactggat tctctccatg gaacagaatc gaggtacaac cagcatcctg ccttccttgc 780
 tgagcattct ctatgtgaaa ctggctgtaa ctgttctcat cgtaggattt gcttttttcc 840
 agaagagaaa ttatttcaga gtgccagaag gctcctgagg agagtggctt gtgggtaaga 900
 tgagatttac caccatctga aagacatctt gtctaccgcy cagcgtgctg agattccgag 960

aagcagccac agaacctact aggaagacaa atctgatgtg gttgtcaatc ctttcaatgg 1020
acctgagtagc ttctataaac ccgagtgagg ttgtgctgga cccaggagcc aggctaggtc 1080
atatatgttg atttttgctg caagacctca tggtttatct acaaacccta aattctttca 1140
cttcagttt taaaactttt ggccaagca ttttatccac agcataacac ctttaaagaa 1200
actctccac ggaaactgct ggttccatgg aatggaaaat tgcaacatgg tttacaagac 1260
agtgc aaacc aagcagcatt ccaagatatg agcttcagaa agttacagga actgtcttgg 1320
gacgagaaag aaggattaaa tagttcccag tccc 1354

<210> 10
<211> 278
<212> PRT
<213> Mus musculus

<400> 10

Met His Ala Leu Gly Arg Thr Leu Ala Leu Met Leu Leu Ile Phe Ile
1 5 10 15

Thr Ile Leu Val Pro Glu Ser Ser Cys Ser Val Lys Gly Arg Glu Glu
20 25 30

Ile Pro Pro Asp Asp Ser Phe Pro Phe Ser Asp Asp Asn Ile Phe Pro
35 40 45

Asp Gly Val Gly Val Thr Met Glu Ile Glu Ile Ile Thr Pro Val Ser
50 55 60

Val Gln Ile Gly Ile Lys Ala Gln Leu Phe Cys His Pro Ser Pro Ser
65 70 75 80

Lys Glu Ala Thr Leu Arg Ile Trp Glu Ile Thr Pro Arg Asp Trp Pro
85 90 95

Ser Cys Arg Leu Pro Tyr Arg Ala Glu Leu Gln Gln Ile Ser Lys Lys
100 105 110

Ile Cys Thr Glu Arg Gly Thr Thr Arg Val Pro Ala His His Gln Ser
115 120 125

Ser Asp Leu Pro Ile Lys Ser Met Ala Leu Lys His Asp Gly His Tyr
130 135 140

Ser Cys Arg Ile Glu Thr Thr Asp Gly Ile Phe Gln Glu Arg His Ser
145 150 155 160

Ile Gln Val Pro Gly Glu Asn Arg Thr Val Val Cys Glu Ala Ile Ala
 165 170 175

Ser Lys Pro Ala Met Gln Ile Leu Trp Thr Pro Asp Glu Asp Cys Val
 180 185 190

Thr Lys Ser Lys Ser His Asn Asp Thr Met Ile Val Arg Ser Lys Cys
 195 200 205

His Arg Glu Lys Asn Asn Gly His Ser Val Phe Cys Phe Ile Ser His
 210 215 220

Leu Thr Asp Asn Trp Ile Leu Ser Met Glu Gln Asn Arg Gly Thr Thr
 225 230 235 240

Ser Ile Leu Pro Ser Leu Leu Ser Ile Leu Tyr Val Lys Leu Ala Val
 245 250 255

Thr Val Leu Ile Val Gly Phe Ala Phe Phe Gln Lys Arg Asn Tyr Phe
 260 265 270

Arg Val Pro Glu Gly Ser
 275

<210> 11
 <211> 813
 <212> DNA
 <213> Mus musculus

<400> 11
 atgcatgctc tggggaggat tccgactttg actttgctga tcttcatcaa tatttttgtg 60
 tctgggtcaa gttgtactga tgagaatcaa acaatacaga atgacagttc atcttctctg 120
 acacaagtta aactacaat gtctgtacag atggataaaa aggctctgct ctgctgcttt 180
 tctagtccac tgataaatgc agtattaatc acatggataa taaaacacag acacctgcct 240
 tctgcacaa tagcatacaa cctagataaa aagaccaatg aaaccagctg cttgggcagg 300
 aacatcacct gggcctccac acctgaccac agtctctgaac ttcagatcag tgcagtggcc 360
 ctccagcatg aggggactta cacatgtgag atagtaacac ctgaaggga ttagaaaaa 420
 gtctatgacc tccaagtgtc ggtgccccct gaggtaacct actttccagg gaaaaacaga 480
 actgcagtct gtgaggcaat ggcaggcaag cctgctgcac agatctcttg gactccagat 540
 ggggactgtg tactaagag tgagtcacac agcaatggca ctgtgactgt caggagcacg 600
 tgccactggg agcagaacaa tgtgtctgtt gtgtcctgct tagtctctca ttcgactggt 660

aatcagtctc tgtccataga actgagtcaa ggtacaatga ccaccccccg ttccttgctg 720
 accattctct atgtgaaaat ggcccttttg gtgattattc ttcttaacgt aggatttgct 780
 ttcttccaga agagaaattt tgccagaaca tga 813

<210> 12
 <211> 270
 <212> PRT
 <213> Mus musculus

<400> 12

Met His Ala Leu Gly Arg Ile Pro Thr Leu Thr Leu Leu Ile Phe Ile
 1 5 10 15

Asn Ile Phe Val Ser Gly Ser Ser Cys Thr Asp Glu Asn Gln Thr Ile
 20 25 30

Gln Asn Asp Ser Ser Ser Ser Leu Thr Gln Val Asn Thr Thr Met Ser
 35 40 45

Val Gln Met Asp Lys Lys Ala Leu Leu Cys Cys Phe Ser Ser Pro Leu
 50 55 60

Ile Asn Ala Val Leu Ile Thr Trp Ile Ile Lys His Arg His Leu Pro
 65 70 75 80

Ser Cys Thr Ile Ala Tyr Asn Leu Asp Lys Lys Thr Asn Glu Thr Ser
 85 90 95

Cys Leu Gly Arg Asn Ile Thr Trp Ala Ser Thr Pro Asp His Ser Pro
 100 105 110

Glu Leu Gln Ile Ser Ala Val Ala Leu Gln His Glu Gly Thr Tyr Thr
 115 120 125

Cys Glu Ile Val Thr Pro Glu Gly Asn Leu Glu Lys Val Tyr Asp Leu
 130 135 140

Gln Val Leu Val Pro Pro Glu Val Thr Tyr Phe Pro Gly Lys Asn Arg
 145 150 155 160

Thr Ala Val Cys Glu Ala Met Ala Gly Lys Pro Ala Ala Gln Ile Ser
 165 170 175

Trp Thr Pro Asp Gly Asp Cys Val Thr Lys Ser Glu Ser His Ser Asn
 180 185 190

Gly Thr Val Thr Val Arg Ser Thr Cys His Trp Glu Gln Asn Asn Val
 195 200 205

Ser Val Val Ser Cys Leu Val Ser His Ser Thr Gly Asn Gln Ser Leu
 210 215 220

Ser Ile Glu Leu Ser Gln Gly Thr Met Thr Thr Pro Arg Ser Leu Leu
 225 230 235 240

Thr Ile Leu Tyr Val Lys Met Ala Leu Leu Val Ile Ile Leu Leu Asn
 245 250 255

Val Gly Phe Ala Phe Phe Gln Lys Arg Asn Phe Ala Arg Thr
 260 265 270

<210> 13
 <211> 2358
 <212> DNA
 <213> Rattus sp.

<400> 13
 agcggaggga tcctt,tcac ggtcaccgct gctcccctac ctgtgaagaq aaagagcacc 60
 gagtgaagccg ctgaaaacca gaaaaccgaa atgctctgct tttggagaac ttctcacgta 120
 gcagtactct tgatctgggg ggtcttcgcg gctgagtcaa gttgtcctga taagaatcaa 180
 acaatgcaga acaattcatc aactatgaca gaagttaaca ctacagtgtt tgtacagatg 240
 ggtaaaaagg ctctgctctg ctgcccttct atttactga caaaagtaat attaataaca 300
 tggacaataa ccctcagagg acagccttcc tgcataatat cctacaaagc agacacaagg 360
 gagacccatg aaagcaactg ctcgacaga agcatcacct gggcctccac acctgacctc 420
 gctcctgacc ttcagatcag tgcagtggcc ctccagcatg aagggcggtta ctcatgtgat 480
 atagcagtac ctgacgggaa tttccaaaac atctatgacc tccaagtgtt ggtgccccct 540
 gaagtaaccc actttccagg ggaaaataga actgcagttt gtgaggcgat tgcaggcaaa 600
 cctgctgcgc agatctcttg gacgccagat ggggattgtg tcgctaagaa tgaatcacac 660
 agcaatggca ccgtgactgt ccggagcaca tgccactggg agcagagcca cgtgtctgtc 720
 gtgttctgtg ttgtctctca cttgacaact ggtaaccagt ctctgtctat agaactgggt 780
 agaggggggtg accaattatt aggatcatc attcaatata tcatcccatc tattattatt 840
 ttgatcatca taggatgcat ttgtcttttg aaaatcagt gctgcagaaa atgtaaattg 900
 ccaaaatcgg gagctactcc agatattgag gaggatgaaa tgcagccgta tgctagctac 960
 acagagaaga gcaatccact ctatgatact gtgaccacga cggaggcaca cccagcgtca 1020

```

caaggcaaag tcaatggcac agactgtctt actttgtcag ccatgggaat ctagaaccaa 1080
ggaaaagaag tcaagagaca tcataattac tgcttttctt tctttaaact tctccaatgg 1140
agggaatta gctcttctga agttcttaga aagcacaat gttctaattg atttgccttt 1200
aagttcttct atcattggaa gtttggaaatc tttgctgcta cctgttaatt ctaggaagaa 1260
ctgatttaat tattacaaag aaagcacatt gttatggtaa aatatcaaatt tgtgcaatac 1320
aatgatgaaa actgagtttc ctcaagaaat aactgcagaa ggaacaatca ttactaaagc 1380
atttcatgtg agttcttcca aaaaagaaaa tccctgtgta tacgacatga ttatggtatg 1440
tgtgtgcctt tatatgtttg ttacaaaatg tgtatatatg cacacatctg attatcaaga 1500
catctctgtc aaaaactcac tggcggtcca gatttatgaa agctaataaa gtgagtattg 1560
gagatgtttt tataatctgta tatgtaaaac tacctcattc tttttaatgg ctacataaaa 1620
ttcatgggtcc ttggatgggc atttagactt tgtgtgtgat gtggtattaa atgataccat 1680
gtggaatggt tcttgtgtg aatctccgca ttatttgagt gcacctgtga gataatttct 1740
gtgagtgtaa tggctctgtc agttggaatg cgcttttatg atcaatagat tagtcaaact 1800
gtgtcagttt acattttctc taattgtgtt taatgtgact tctccatat tcttattctt 1860
atgtttttaa tatcttctc ttacaccttt atactttcca tctttaatta accagttggg 1920
tgatgtgtct taaggttgtg atattaactt tattatttaa tggaactgga ttcatatctt 1980
tggttttcat gtccacaaaa gagatagaaa gcatttgtaa agacagtatt ttcaactcct 2040
tgtattacta caaaaatggt gacatctgat gcacaacagt tttatggatg ttatgaattg 2100
tgtgttttta acattctatt ctgatgtact tataagagag caactgtctt tgaactatat 2160
atgtagggtg gagaacttgg agtactttat gtgctaatag gatggtaatg ggatgatata 2220
acttttccct ccagtttttt ggagggaaat attaggaata catgtattga taatttttag 2280
catatatatt ttaattgtta aaaataaacc tgttcccttt atatcaggaa agatattaaa 2340
aatggattta ttcattctc 2358

```

<210> 14
 <211> 327
 <212> PRT
 <213> Rattus sp.

<400> 14

Met Leu Cys Phe Trp Arg Thr Ser His Val Ala Val Leu Leu Ile Trp
 1 5 10 15

Gly Val Phe Ala Ala Glu Ser Ser Cys Pro Asp Lys Asn Gln Thr Met
 20 25 30

Gln Asn Asn Ser Ser Thr Met Thr Glu Val Asn Thr Thr Val Phe Val
 35 40 45

Gln Met Gly Lys Lys Ala Leu Leu Cys Cys Pro Ser Ile Ser Leu Thr
 50 55 60

Lys Val Ile Leu Ile Thr Trp Thr Ile Thr Leu Arg Gly Gln Pro Ser
 65 70 75 80

Cys Ile Ile Ser Tyr Lys Ala Asp Thr Arg Glu Thr His Glu Ser Asn
 85 90 95

Cys Ser Asp Arg Ser Ile Thr Trp Ala Ser Thr Pro Asp Leu Ala Pro
 100 105 110

Asp Leu Gln Ile Ser Ala Val Ala Leu Gln His Glu Gly Arg Tyr Ser
 115 120 125

Cys Asp Ile Ala Val Pro Asp Gly Asn Phe Gln Asn Ile Tyr Asp Leu
 130 135 140

Gln Val Leu Val Pro Pro Glu Val Thr His Phe Pro Gly Glu Asn Arg
 145 150 155 160

Thr Ala Val Cys Glu Ala Ile Ala Gly Lys Pro Ala Ala Gln Ile Ser
 165 170 175

Trp Thr Pro Asp Gly Asp Cys Val Ala Lys Asn Glu Ser His Ser Asn
 180 185 190

Gly Thr Val Thr Val Arg Ser Thr Cys His Trp Glu Gln Ser His Val
 195 200 205

Ser Val Val Phe Cys Val Val Ser His Leu Thr Thr Gly Asn Gln Ser
 210 215 220

Leu Ser Ile Glu Leu Gly Arg Gly Gly Asp Gln Leu Leu Gly Ser Tyr
 225 230 235 240

Ile Gln Tyr Ile Ile Pro Ser Ile Ile Ile Leu Ile Ile Ile Gly Cys
 245 250 255

Ile Cys Leu Leu Lys Ile Ser Gly Cys Arg Lys Cys Lys Leu Pro Lys
 260 265 270

Ser Gly Ala Thr Pro Asp Ile Glu Glu Asp Glu Met Gln Pro Tyr Ala
275 280 285

Ser Tyr Thr Glu Lys Ser Asn Pro Leu Tyr Asp Thr Val Thr Thr Thr
290 295 300

Glu Ala His Pro Ala Ser Gln Gly Lys Val Asn Gly Thr Asp Cys Leu
305 310 315 320

Thr Leu Ser Ala Met Gly Ile
325